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In The

Supreme Court of the United States

October Term, 1998

RANDON BRAGDON, D.M.D.,

Petitioner,

v.

SIDNEY ABBOTT, et al.,

Respondents.

On Petition For A Writ Of Certiorari To The United States Court Of Appeals For The First Circuit

BRIEF OF THE *AMICUS CURIAE* CLINICAL RESEARCH ASSOCIATES IN SUPPORT OF RANDON BRAGDON, D.M.D., PETITIONER

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STATEMENT OF INTEREST OF *AMICUS CURIAE*

Clinical Research Associates hereby submits this brief as *amicus curiae* in support of Dr. Randon Bragdon, D.M.D.[1] The brief is submitted by the written consent of all parties to the suit.

Clinical Research Associates, known worldwide as CRA, was initiated over 23 years ago by dental clinicians to test the efficacy and clinical usefulness of products they use in dental treatments. CRA is a unique non-profit research organization comprised of 45 on-site basic scientists, engineers, and support staff, plus over 400 dental clinicians located in 18 countries who volunteer their time and expertise to field test products within their practice environments. CRA's activities have been compared to Consumers' Union and the Consumers' Report publication, although CRA works only with products used in dental treatments. CRA performs about 20,000 product evaluations each year, working with about 650 different products secured from the worldwide market. Results of CRA's work are published monthly in nine languages and circulated to 72 countries.

Since its inception, CRA has worked actively with infection control products, including over 60 different face-mask brands, 36,000 gloves, 20 different types and styles of protective clothing, over 150 different environmental surface disinfectant formulations, 25 instrument immersion disinfectants, 15 commercial heat sterilizers,

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[1] Pursuant to Supreme Court Rule 37.6, this brief was prepared entirely by counsel for CRA. No monetary compensation was received from any other person or entity.

12 different designs of air purification equipment, and over 40 different products sold for treatment of dental unit waterlines. CRA is the only place in the world where all types of infection control products used in dentistry receive extensive efficacy testing routinely.

CRA's mission and only purpose is to test products used in dental treatments for efficacy and clinical usefulness, and to communicate to the dental community all results.

SUMMARY OF ARGUMENT

Throughout the proceedings of this case in the lower courts, the following key issue has failed to receive consideration: Efficacy of the "universal precautions" concept, claimed by experts to protect clinicians and patients against significant risk of transmission of infectious diseases, is *flawed seriously* by the fact that it is totally dependent on the efficacy of *products* used to implement it (face-masks, operating gloves, disinfectants, sterilizers, etc.). CRA's lab has tested thousands of products used for infection control to support the universal precautions concept over the past 23 years, and has demonstrated clearly and repeatedly that most of these products available worldwide fail to meet their intended purposes. *Failure of infection control products that support the universal precautions concept virtually negates the concept.* Although there may be a visual appearance of protection, if masks fail to filter properly, operating gloves leak, and disinfectants and sterilizers fail to kill as expected, this places both clinicians and patients at significant risk every time

infectious organisms are present. Furthermore, efficacy of *all* the products, efficacious or not, needed to protect clinicians and patients from infectious disease transmission is easily compromised by clinical mistakes and accidents, which can occur unexpectedly at any time and inflict a lifetime of serious ill health and/or untimely death. (*See* reference # 1, pages App. 1-4).[2]

ARGUMENT

THE UNIVERSAL PRECAUTIONS CONCEPT DOES NOT ALLEVIATE SIGNIFICANT RISK TO CAREGIVERS DURING TREATMENT OF INFECTIOUS PATIENTS BECAUSE THE PRODUCTS NECESSARY TO IMPLEMENT THE CONCEPT OFTEN FAIL THEIR INTENDED PURPOSE

Because microbes are invisible, clinicians cannot determine by themselves the efficacy of the infection control products they use. They have no way to test for face-mask filtration capacity, operating glove leakage, and disinfectant and sterilizer kill. Ideally, efficacy of these critical products should be confirmed before each patient treatment, but no tests exist anywhere that provide this capability. CRA has demonstrated that product cost cannot be used as an indicator of product efficacy. So this leaves clinicians totally at the mercy of the product manufacturer's integrity. All the above factors open

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[2] All articles referenced herein are reproduced in the Appendix to this brief, noted by reference number and page number.

opportunities for extensive deception and fraud in product promotional claims.

Today none of the agencies originally entrusted with safeguarding the public against non-efficacious products used in healthcare such as the EPA, FDA, CDC and American Dental Association, actually *test* the products they certify or recommend. Although doubts about disinfectant efficacy have been pointed out by the General Accounting Office (GAO), (*See* reference # 2, pages App. 5-13), lack of government funding for a central testing lab allows the problem to continue unabated.

Today, after many years without non-biased confirmatory testing there now exists a large number of products promoted as protective against transmission of infectious microbes that, in fact, do not fulfill that purpose. Clinicians purchase and use these products routinely, hoping they are protected. The authors of the universal precautions concept and the experts who have testified in this case that no precautions should be taken by caregivers beyond those prescribed by the universal precautions concept have all *assumed* that the products that support the concept perform as expected. This means the products are assumed to block microbes or kill microbes before they enter the clinician's body.

CRA's work demonstrates that these assumptions are ill founded. In actuality, every time a person with an infectious disease is treated, the clinician plays Russian roulette with his own health, and his life, as well as his attending staff and subsequent patients.

This brief is supported by data from studies performed by CRA and others, included in the appendix

hereto, which clearly demonstrate that this *blind faith* in the protection afforded by infection control products (universal precautions) is *not justified.*

Of critical importance in the instant case, the data demonstrate the protective measures commonly referred to as universal precautions do *not* provide the protection claimed by the authorities and experts referenced in the testimonies cited in this case. Unfortunately, the experts whose testimony was presented by Respondents in the proceedings before the First Circuit on remand[3] are not microbiologists actually involved in testing efficacy of infection control products.

It appears the experts based their testimonies on the assumption that large numbers of healthcare providers are not believed to have become HIV positive from work-related exposures. This does not mean there is not significant risk to caregivers using the universal precautions concept. There are cases on record where experts have been frustrated and perplexed because serious transmissions occurred in the presence of all the proper universal precautions. These cases point clearly to the fact that universal precautions strategies can, and do, fail to protect both clinicians and patients. (*See* references # 3 & 4 on pages App. 14-40).

Every breach does not elicit an infection, as the referenced paper illustrates. Although the hepatitis B infected

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[3] This testimony is contained in CDC's 1987 *Recommendations for Prevention of HIV Transmission in Health-Care Settings,* 36 MMWR No. 2S (Aug. 21, 1987), as referenced in Dr. Bragdon's brief at pages 4-5.

surgeon caused disease in 18 patients, another 124 he treated did not contract the disease. This is because a whole set of circumstances generally must coincide at the moment of organism transfer in order for the infection to result in subsequent disease. These circumstances are thought to include, but are not limited to, such factors as: (1) virulence of the microorganism at the time of transfer, (2) numbers of the microorganism transferred, and (3) the immune status of the recipient at the time of transfer. Hence, in every case, *chance* plays a part in whether or not infection will escalate to disease. Indeed, the word "disease" is defined as "failure of the *adaptive mechanisms* of an organism to counteract adequately the stimuli or stresses to which it is subject, resulting in disturbance in the function or structure of any part . . . " (Blakiston's Pocket Medical Dictionary) (4th ed. 1976) (emphasis added). Therefore, it needs to be understood from the beginning that determination of "significant risk" is more than a numbers game of how many infected people constitute "significance." It is, more importantly, a determination of the importance of each human life suddenly affected by chance when a face-mask or glove leaks infectious materials, or a disinfectant or sterilizer fails to kill critical microbes, or a needle or other sharp instrument penetrates barriers. In truth, determination of significant risk pits the life of the infected patient against the life of the uninfected caregiver, and the court is charged with the responsibility to choose which is to be favored.

CRA submits the following data as evidence that the products used to implement the universal precautions concept cannot be relied upon to alleviate the significant

risk always present when clinicians treat patients with infectious diseases:

1. **Face-Mask Efficacy (*See* reference # 5 on pages App. 41-56).**

When 42 different face-masks and 2 face shields used by dental clinicians in the U.S. were tested for ability to block aerosolized bacteria, only 22 of the 42, or about half (52%), passed this test. In further tests with the 42 masks, when blue dye was aerosolized to simulate microbe laden aerosols generated during dental procedures, and a human subject within a sealed chamber breathed the dye laden aerosols, *all* the masks (100%) showed significant leakage through to the mask interior, and *only one* of the 42 masks was designed in such a way that it could be adapted at its border to fit securely enough to block dye penetration at this critical interface. We have since found that all face-masks sold worldwide to dental clinicians become overloaded quickly (within 10 to 30 minutes) and allow material to penetrate to the mask interior, and only two masks are designed that prevent ingress of materials at the periphery.

This penetration of debris aerosolized from the patient's oral cavity is highly significant because it brings infectious organisms from another person's body into direct contact with the lips, nostrils, and skin, and into the respiratory passages. This affords the possibility for *direct contact of critical mucous membrane tissues* with infectious material.

Current mask designs do not provide any way for clinicians to determine when leak-through begins or the

amount present. Therefore, as masks entrap and fill up with aerosolized material, and leak to the mask interior, the mask becomes a source of, rather than a barrier to, aerosolized infectious microorganisms.

Dental handpieces and their cutting drills used to perform the tooth drilling emit large amounts of air and water under pressure while rotating at speeds up to 250,000 to 300,000 rpm. These instruments cause massive aerosolization of patients' oral tissues. Research confirms that about 50% of the aerosols generated during dental procedures are three µm and smaller in size. Such particles remain suspended at the end of treatment for long time periods, creating risk even after treatment of infectious patients is completed. Furthermore, three µm and smaller aerosols can penetrate the human respiratory system all the way to the alveoli of the lower respiratory tract, which greatly increases infectivity. Although the experts in this case claimed that they know of no cases where HIV has been transmitted by aerosols, in dental applications the possibility of such a transmission cannot be denied. It is known that HIV particles can be present in saliva. It is known that saliva is aerosolized during use of dental handpieces, ultrasonic scalers, air polishers, and in many other less obvious dental treatments. It is known that face-masks do not present an impenetrable barrier. These facts lead to the conclusion that risk is present, and transmission of HIV to alveolar macrophages and other key cells within the lungs is possible during dental procedures.

CRA submits that the referenced data show face-masks do not provide the protection expected by dental clinicians from infectious saliva, blood, pus, tooth debris

and other materials aerosolized during dental treatment, and they leave clinicians subjected to significant risk of disease transmission while creating the illusion of protection.

2. Latex Examination Glove Efficacy – (*See* references # 6-10, pages App. 57-84).

Over its 23 years of operation, CRA has tested over 36,000 clinical operating gloves made of latex, vinyl, nitrile, and other non-latex materials. If we consider just the gloves made of latex, since these are the most used, CRA has tested 84 different brands manufactured in the U.S., Malaysia, Taiwan, India, China, and Thailand. If we combine the pinhole data[4] for latex gloves from just the test results published in the enclosed references, and do not include any other reports, we find an overall prevalence of 6.75% pinholes in the glove brands tested. (*See* references # 6-9, pages App. 57-77.) Most pinholes are not visible to the naked eye, but can be disclosed by filling the glove with air and submerging it in water, by filling the glove with water and looking for seepage of the water to the external after a few minutes, by viewing the glove under a scanning electron microscope, or by rubbing gloved hands of humans with dye and then looking for dye spots on the hands of the person after degloving.

Most dentists treat about 15 patients per day and work about 240 days per year. The 6.75% pinholes found

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[4] A pinhole is defined as a small breach in the integrity of the glove material generally resulting from manufacturing problems.

by CRA allows *the average dentist to use about 486 gloves in a year that have holes in them* that permit the patient debris, which the dentist wears gloves to avoid, to leak through the gloves and onto their hands. Using the 4% pinhole maximum allowed by the FDA for latex examination gloves, a dentist would use 288 gloves in a work year that leaked.

All of CRA's glove integrity test data above is the result of testing new, unused latex examination gloves. Logically, clinical use stresses latex gloves, and causes one to suspect an increasing number of breaches in this barrier as use time and work challenges increase. Tests performed at Johns Hopkins University School of Nursing (*See* reference # 10, pages App. 78-84) showed a volume increase in fluid leaked by 240 latex gloves tested of 0.08 µl in new unused gloves vs. 2.20 µl in gloves subjected to simulated clinical procedures. This represents more than a 27-fold increase in material leaked due to usage. So not only must clinicians beware of leakage of new, unused gloves, but they must expect significantly more leakage as they move through routine procedures. And it is self-evident that latex gloves are easily penetrated by sharp instruments. It is also evident that it is not necessary to be exposed to a large quantity of infected blood to become HIV positive following an accident (*See* reference # 1, pages App. 1-4).

What number of glove leaks constitutes a "significant risk," within the meaning of 42 U.S.C. § 1282(b)(3)? CRA submits that the referenced data show latex examination gloves do not provide the protection expected by dental clinicians from infectious saliva, blood, pus, tooth debris, soft tissue, and other materials handled during dental

treatment, and they leave clinicians subjected to significant risk of disease transmission while creating the illusion of protection.

3. Environmental Surface Disinfectants (*See* references # 11-15, pages App. 85-160).

Environmental surface disinfectants are liquids used on a vast variety of objects in homes, offices, hospitals, restaurants, hotel rooms, and dental operatories. They are used on all types of surfaces ranging from toilet seats and bowls, kitchen sinks and counters, and walls and floors everywhere, to objects in dental operatories and hospital operating rooms.

The wide variety of applications and uses has inspired the creation of a large number of products, which unfortunately do not perform equally well. Yet to most people, any container labeled "disinfectant" is presumed to kill everything, instantly and completely. This perception represents a gross misunderstanding of the chemistry of disinfectants and the physiology of microorganisms, and can cause serious mistakes to be made by those who select the disinfectants to be used in clinical treatment areas, exposing the clinical personnel to significant risk.

Although the potential of contaminated environmental surfaces to transmit diseases has been questioned, confirmation of the fact that organisms are not rendered non-viable immediately upon leaving the body has always made it impossible to rule out environmental surfaces as fomites. In addition, experiments designed

specifically to investigate this point have repeatedly demonstrated transmission of both infection and disease. For example, in experiments using human hands contaminated with hepatitis A virus, it was shown clearly that the virus was transferred from hand to hand, from hands to objects, and from objects to hands (*See* reference # 11, pages App. 85-108). In other experiments, healthy human volunteers were infected with colds by handling tiles and cup handles contaminated by the investigators with rhinovirus (*See* reference # 12, pages App. 109-118). While CRA is unaware of similar experiments using HIV, experiments by competent researchers experienced in handling HIV reported viable virus still present after five minutes of exposure to a 10% dilution of household bleach. (*See* reference # 13, pages App. 119-124).

This indicates that HIV is relatively resistant, at least to the 1:10 bleach, which has been recommended as the environmental surface disinfectant of choice by the CDC for many years.

Since clinicians perceive all disinfectants as equally effective, they shop for formulations that are cosmetically pleasing (agreeable fragrance, does not corrode or stain, visually pleasing packaging, etc.), and never stop to consider microbial kill. This is done under the misplaced assumption that consumers are protected from ineffective disinfectants by EPA and FDA regulations. Although these agencies have the authority to control which disinfectants are sold within the U.S., neither performs confirmatory tests for efficacy and both lack a simple and clear communication system to inform consumers about what the disinfectant can and cannot do. These problems have resulted in use of disinfectants that do not kill

properly and leave viable organisms intact and able to infect people in most of the medical and dental facilities throughout the U.S.

Of 39 environmental surface disinfectants commonly used by dental clinicians and tested by CRA in 1988-89, only three, or 8% passed all the tests. (*See* reference # 14, pages App. 125-158). This means 92% of 39 products could not kill a clinically representative, resistant virus (poliovirus) and bacteria (the tuberculosis bacteria, *Mycobacterium bovis*) in both the absence and presence of human blood.

Five years later after packaging, ownership, and formulation changes by many disinfectant manufacturers, CRA revisited this work and added some new formulations. So far, 59 formulations have been tested in this second round and only seven have passed all the tests, (*See* reference # 15, pages App. 159-160), which means 88% still fail to kill under clinically relevant conditions.

Of the six disinfectant *active ingredients* used worldwide, only two provide broad spectrum disinfection, and even these must be used at specific dilutions and/or in specific combinations with certain other chemicals, or they also fail to kill. Ethyl alcohol at about 70% by weight and household or institutional bleach diluted no more than 1:2 were the only formulations that provided reliable kill, regardless of the test method, test organism, or contact time used, both in the absence and presence of fresh human whole blood.

CRA submits that the referenced data show that most environmental surface disinfectants do not provide the microorganism kill expected by dental clinicians, and

they leave clinicians subjected to significant risk of disease transmission while creating the illusion of protection.

4. Instrument Immersion Disinfectants (*See* references # 16-17, pages App. 161-170).

Formulations of instrument immersion disinfectants can differ from environmental surface disinfectants, so they are considered separately here. Glutaraldehyde (ideally at dilutions of about 2% or 3%) has been the worldwide standard for instrument immersion for the past ten-plus years. It should not be used for environmental surfaces because it emits fumes that can be toxic when it is used liberally on countertops in unventilated areas, it can elicit skin sensitivities, and it generally has a slow kill of the tuberculosis bacteria that would extend surface contact times beyond all practicality. However, as an instrument immersion disinfectant its characteristics of reliable broad spectrum kill, even in the presence of gross amounts of bodily contaminants such as blood, feces, tissue, etc., make it a very unique and valuable clinical product.

However, even glutaraldehyde disinfectants are not foolproof. In 1991, when CRA tested 12 different formulations (sold under 12 different brand names), water dilutions of the disinfectants suggested on the labels proved to be a significant problem. (*See* references # 16-17, pages App. 161-170). Of 15 recommended dilutions, 14 (93%) required *more time* than stated on labels to kill poliovirus and the TB bacteria. Five required *hours* longer than the times stated, with two requiring *five hours* longer than

times stated on the labels. Since 1991 when CRA performed this work, FDA has attempted to correct this problem and has eliminated the worst discrepancies, but some problems still exist with kill-time label claims on some products. Ironically, if clinicians are willing to allow any amount of time, up to six hours, for disinfection to take place, CRA data showed all the products tested eventually killed the test organisms adequately.

CRA submits that even the most potent disinfectants, known as "high level disinfectants", now regulated under the auspices of FDA, have not provided protection expected by clinicians, leaving them subjected to significant potential for disease transmission, while creating the illusion of protection.

5. Sterilization Equipment (*See* reference # 18, pages App. 171-184).

Heat sterilization equipment is one area of infection control products where governmental control (FDA clearance) has been very strict, and proof of a substantial margin of safety has been required. However, the decision to "grandfather" some sterilizers sold before 1976, and still sold today, presents problems because they have not been subjected to the same performance criteria.

However, the biggest problem in the dental sterilizer market is dental drill sterilization. It has been shown that dental drills generally require a little more time to render them sterile than the conventional hand instruments, such as mouth mirrors and "picks" used by dentists. Therefore, FDA requires a separate submission and confirmatory data for dental drill sterilization.

Unfortunately, dental clinicians are totally ignorant of this requirement. Clinicians purchase and use their sterilizers for all instruments, including dental drills. There is no realization by clinicians that the dental drills may require a longer time to be rendered sterile.

In the summer of 1998, a researcher shocked the dental community by reporting that several models of chemical vapor sterilizers did not render dental drills sterile. (*See* reference # 18, pages App. 171-184). He demonstrated lack of sterility by recovering viable test spores within the dental drills. CRA then examined the U.S. dental sterilizer market and found that five of ten popular dental sterilizers (50%) had not received 510K clearance for handpiece sterilization, even though manufacturers and distributors of these sterilizers knew that dental clinicians were likely to use the sterilizers to treat dental drills.

Therefore, sterilization times for dental drills processed in the five sterilizers yet to be cleared are unknown. The clinical risk related to this fact can only be surmised.

Because of the significant risk that exists under the universal precautions protocol, at a minimum Dr. Bragdon should have been allowed to present evidence such as that contained herein to the finder of fact. The First Circuit erred in affirming the summary judgment in favor of Respondents.

CONCLUSION

CRA has addressed the term "significant risk" as it is used in the Americans with Disabilities Act related to dental clinical treatment of HIV positive and AIDS patients. Data and comments presented are meant to address testimonies of experts in this case who declared that no protective measures beyond those known as "universal precautions" are necessary to secure dental clinicians against transmissions during treatment. CRA has submitted a body of data generated by our lab and others to show that the products necessary to implement the universal precautions concept (face-masks, latex gloves, disinfectants, and sterilizers) are defective even when fresh, new and unused. CRA submits that when the products fail to block or kill as expected, the universal precautions concept is negated, and clinicians who operate thereunder are exposed to significant risk. The situation is analogous to requiring every infantry person to carry into battle a rifle that is known to have a defective trigger that allows the gun to be fired sometimes, as expected, but jams sometimes without warning. Would anyone disagree that the soldiers were at risk of getting shot and maybe killed when their gun jammed and the enemy got off the first shot?

To place people infected with a virus transmitted by their blood and other body fluids into routine mainstream healthcare, and then disallow any special precautions simply invites catastrophic incidents from time to time, without any way to predict exactly when a caregiver will become infected. The nature of viral transmission is generally haphazard unless the virus is particularly virulent and/or the immunity pool is nonexistent.

Therefore, all exposure incidents may not lead to infection and disease, and the courts may be tempted to play a numbers game to determine significance of the risk. The unfortunate part of this approach is that the infection is exquisitely significant to the caregiver infected, and possibly also to the index patient involved in the transmission. The point is, we now have *two* infected individuals instead of just the original one. If we wanted to play a numbers game, we could say we just doubled the original number of infected people, and doubling of a number is considered a significant increase to most people.

CRA's data indicate there *is* significant risk, not overcome by universal precautions, every time HIV positive patients receive treatment where bleeding tissue and use of sharp instruments is involved.

Is it safer for dentists to treat HIV positive patients in a hospital? The answer to this question is both yes and no. No, it probably does not make a difference to the safety of the infected patient. YES, it could be safer to the uninfected clinicians because, in the event of a clinical accident, within the hospital they can receive *immediate* prophylactic treatment. Prophylactic treatment involves administration of drugs not readily available just anywhere. Ideally, post-exposure drugs should be administered immediately after exposure to allow the best chance of effectiveness. In suburbs and rural areas, after accident, clinicians may have to lose precious time traveling to centers where these drugs are available. In some cases, the travel could involve a delay of many hours, which could diminish chances of a positive outcome.

Analysis of Dr. Bragdon's petition should *focus on control of infectious diseases*. While unfounded discrimination is improper under the ADA and within our society, when there exists a significant threat to the life of a caregiver the law should not prevent the caregiver from taking all reasonable precautions. The data presented herein show that significant risk exists every time clinicians treat an infected patient using the defective defenses of the universal precautions protocol. This risk is escalated exponentially when an accident or mistake occurs that causes further compromise of universal precautions products, which are highly questionable from the onset.

Theoretically, the products that support the universal precautions concept should provide the protection sought, in the absence of any accidents that compromise them. But, in actuality, the infection control products provide no more than a *show* to reassure patients that the personnel treating them are aware of, and concerned about, transmission of diseases, and to reassure clinicians by placing a shield or barrier between their body and infectious materials. But any thinking clinician realizes the protection is weak and minimal and easily penetrated in a split second of an unfortunate move. Most clinicians and patients (and, for that matter, the experts who testified in this case), are *unaware* of the flaws in masks, gloves, disinfectants, and sterilizers, which we have reported here. Based on the evidence herein, to say or imply that universal precautions products are enough to remove significant risk of viral transmission during treatment of HIV positive patients is wishful thinking at best,

and reckless disregard for the health and lives of caregivers and other patients at worst.

Respectfully submitted,

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REFERENCE NO. 1

The Boston Globe – Tuesday, April 6, 1999

A nurse hopes her infection will spur action

By Richard A. Knox
Globe Staff

Nurse Karen Daley was shoving a used hypodermic needle into a disposal box at the Brigham and Women's Hospital emergency room last July when she felt a sharp pain. A used needle inside the box was pointing upward, and it stuck her gloved finger.

Months later she got the awful news: The accidental needle-stick injury had infected her with the viruses that cause AIDS and hepatitis C, a serious and often fatal liver disease.

US health-care workers report about 600,000 accidental injuries from needles and other "sharps" each year, resulting in about 1,000 serious infections. What made Daley's case unique is that she is president of the Massachusetts Nurses Association.

Ironically, the association was already preparing legislation that would require hospitals to use the best available needle stick prevention technology.

To further the cause, the 46-year-old nurse plans to reveal her infection publicly today in Beacon Hill testimony.

"She's one amazing person," said Gloria Craven, the association's lobbyist. "She's saying 'How can we turn this into something good?'"

Daley declined to speak about her situation before her testimony, but colleagues say she is coping with stoicism and determination. She reportedly suffers serious fatigue from her infections.

Through June of last year, the US Centers for Disease Control recorded 54 cases of documented HIV infection among health-care workers and 133 possible cases. Nurses led the list of workers with occupationally acquired HIV infection.

It is rare for victims of accidental needle sticks to become infected with both hepatitis C and the human immunodeficiency virus (HIV), which causes AIDS. Both are potentially fatal, and hepatitis C is the leading cause of liver failure requiring transplantation.

The dual infection complicates Daley's treatment, a nurse colleague said, and makes it difficult to predict her course.

Although details of Daley's injury were unavailable yesterday, it may have been impossible to identify the patient who was the source of her infections. The disposal box presumably contained needles for a variety of patients treated that day.

One of Daley's colleagues called her injury "100 percent preventable" by such means as better-designed disposal boxes, more frequent emptying of such receptacles, and needles that are covered by a retractable plastic

sheath except at the moment when they are in contact with a patient's skin.

"Overall the health-care industry has not seen fit to provide the devices that would make the work environment safer for nurses and others," said Evelyn Bain, an occupational safety and health specialist at the nurses association.

The costs of safer technology are far outweighed, Bain said, by the costs of investigating needle-stick incidents and providing prophylactic drugs costing $1,300 to $1,800 per case, not to mention the future treatment costs and human suffering if a health-care worker becomes infected. "One local hospital that reduced needle-stick injuries by 50 percent with a prevention program still recorded 101 injuries in 1997," she added. "That facility spent $141,000 to follow the injured workers."

The proposed legislation, which will be considered by the Legislatures's Joint Health Care Committee today, would set up a mechanism within the state health department requiring health-care facilities to employ best-available prevention strategies.

"Facilities would have to do only three things: purchase the appropriate equipment; look at how to prevent injury; and when an injury occurs, document it and keep a log so we can use it for quality improvement," Craven said.

So far Oregon is the only state to pass such a law, she said.

Judy Glasser, a spokeswoman for the Massachusetts Hospital Association, said hospitals generally support the

proposal. "We believe it moves in the right direction, and we believe we should do everything we can to protect our employees and caregivers," Glasser said.

Staff members at the nurses association said they learned only about a month ago about Daley's infections, which the administration of prophylactic drugs had failed to prevent. "Most of us haven't stopped crying yet," one said.

Daley's career may not be over, but her 25 years of direct patient care may be at an end because of the risk she may pose to patients. About 85 percent of individuals infected with hepatitis C virus become chronic carriers, and so far no treatment has been shown to eliminate HIV from the tissues of infected individuals.

REFERENCE NO. 2

United States General Accounting Office
Report to Congressional Requesters
August 1990

DISINFECTANTS: EPA Lacks Assurance They Work

GAO/RCED-90-139

Executive Summary

Purpose

Disinfectants – about a $1 billion per year market – are used to kill germs on inanimate surfaces and objects in hospitals, schools, restaurants, and homes. Because users cannot see whether disinfectants kill bacteria, fungi, and viruses, the use of ineffective disinfectants poses a threat to public health and wastes consumer dollars.

Mounting concerns about whether hospital and household disinfectants work as claimed and the adequacy of the Environmental Protection Agency's (EPA) disinfectants program led the House Committee on Government Operations to request that GAO review EPA's regulation of the efficacy of disinfectants.

Background

Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), EPA generally must register (license) pesticide products, including disinfectants, before they

are marketed. EPA may register a pesticide product only if EPA determines that it is effective, when used as claimed, without causing an unreasonable risk to health or the environment. For most pesticides, EPA allows the marketplace to regulate product performance (efficacy) because users can see whether the pesticide is effective against the target pest. However, registrants of disinfectants intended to protect public health must submit efficacy data to substantiate each product performance claim and use.

Until 1982, EPA conducted limited preregistration confirmatory and post-registration enforcement tests on disinfectants at its laboratory facilities in Beltsville, Maryland. EPA discontinued disinfectant testing in 1982 primarily because of budget constraints. Currently, EPA relies on its review of registrant-submitted efficacy data to register disinfectants. As of September 1, 1989, about 4,100 disinfectants for public-health use were registered with EPA, representing about 18 percent of approximately 23,000 registered pesticide products.

Results in Brief

EPA does not know whether disinfectants kill the germs claimed on product labels for four reasons. First, although the validity of

methods and performance standards used to assess the efficacy of disinfectants has been the source of scientific controversy for over a decade, EPA does not independently test disinfectants before registering them and lacks criteria to assess the validity of registrant-proposed test methods and modifications. Second, EPA has made little progress in resolving these controversies because of budget constraints and inadequate research management. Third, EPA lacks sufficient internal controls to ensure the quality and integrity of the data that registrants submit on disinfectant efficacy. Fourth, EPA lacks an enforcement strategy to ensure that, once registered, disinfectants sold and distributed in the marketplace work as claimed.

The extent to which ineffective disinfectants are marketed is unknown. Although the scientific controversies cloud the issue somewhat, evidence from EPA, the states, and others suggests that up to 20 percent of disinfectants on the market may be ineffective.

Principal Findings

Validity of Test Methods

EPA lacks assurance that the test methods and performance standards

used by registrants to substantiate disinfectant efficacy claims are valid. EPA primarily relies on standard-setting organizations, such as the Association of Official Analytical Chemists (AOAC) and the industry itself to develop test methods and performance standards. However, these methods and standards have been embroiled in scientific controversies for over a decade. For example, scientists have debated whether the AOAC Use-Dilution Method, the most widely used test method, is reproducible, accurate, and precise, and whether the performance standard (pass/fail criterion) established by EPA is valid. Although EPA believes that the existing methods and standards are acceptable for registering and enforcing disinfectant efficacy claims, the controversies have impaired the credibility of the disinfectant program. An ad hoc industry/state group recently has developed a test method to replace the AOAC Use-Dilution Method and is expected to present the results of its research to the AOAC in September 1990. EPA officials believe that the new method is reproducible and reliable and will consider whether to require that disinfectants be retested using it after AOAC considers it for adoption.

EPA has contributed to the controversies by accepting test methods

and modifications without criteria and independent laboratory data for evaluating their validity. For example, EPA accepts three different test methods to demonstrate that disinfectants kill tuberculosis bacteria. At least one product tested under two of the methods produced substantially different results. Although EPA has registered the product on the basis of one of the methods, EPA lacks the laboratory information needed to explain the differences in results between the methods.

Although EPA has been aware of the scientific controversies for years, it has made little progress in resolving them because of problems in conducting needed research. EPA's 6-year, $384,000, cooperative agreements with the University of North Carolina did not fulfill EPA's research objectives to improve disinfectant efficacy methods because EPA inadequately managed the agreements. EPA has also made little progress in conducting additional research because of budget constraints. In April 1990, EPA announced that it would spend $600,000 for research on certain disinfectant efficacy methods and estimated an additional $1.2 million will be needed.

Controls Over Quality and Integrity of Data

To ensure the quality and integrity of registrant-submitted disinfectant data, EPA reviews the data prior to registration and performs laboratory inspections and data audits. GAO, however, found internal control weaknesses in these programs. For example, EPA has not inspected the majority of labs that have performed disinfectant efficacy studies. In fact, EPA was aware of only 12 of the 92 labs that had performed these studies. Although these programs need to be improved they, in themselves, are not an adequate substitute for a preregistration program to selectively test disinfectant efficacy by an independent laboratory. Data reviewers, lab inspectors, and data auditors generally cannot identify cases in which registrants have selectively submitted data indicating that their disinfectants work because they generally do not observe the tests in progress and no physical evidence remains from the tests conducted.

Monitoring and Enforcement of Marketed Disinfectants

EPA's registration process by itself cannot provide assurance that disinfectants are effective because registrants could market ineffective batches, either intentionally or inadvertently, after registering them.

However, EPA does not enforce the efficacy claims of disinfectants on the market. EPA discontinued its limited enforcement testing program in 1982 primarily because of budget constraints. Since 1982, EPA has looked to the states, user groups, and the industry to enforce efficacy claims. However, GAO found few states and no users monitoring disinfectant efficacy because of cost concerns. Only five states test disinfectants for efficacy, and these states have limited programs. Moreover, EPA lacks a strategy to channel complaints about potentially ineffective disinfectants from the states, user groups, and the industry and to take appropriate enforcement action against disinfectants found to be ineffective. Although EPA needs to resolve the scientific controversies that surround disinfectant efficacy test methods and performance standards, these controversies should not prevent EPA from developing an enforcement strategy, in conjunction with the states, user groups and industry, to ensure that marketed disinfectants work as claimed. Public health and consumer welfare may be comprised without such assurance.

Recommendations

GAO is making recommendations to the Administrator, EPA, to correct deficiencies and restore credibility

in the disinfectant program, including (1) developing a plan to resolve the scientific controversies that surround disinfectant efficacy test methods and performance standards; (2) developing and publishing a policy that establishes criteria for evaluating the validity of new test methods and modifications, including criteria for determining when independent laboratory data are needed for validation; (3) improving internal controls over its current programs to ensure the quality and integrity of registrant-submitted efficacy data and conducting preregistration tests to selectively verify registrant claims; (4) establishing an enforcement strategy in conjunction with the states, user groups, and industry to ensure that marketed disinfectants work as claimed; and (5) preparing a cost-benefit analysis of alternatives for a laboratory facility to research and test the efficacy of disinfectants, including the option of charging fees to register disinfectants to help finance such a facility.

Agency Comments

GAO did not obtain official agency comments on this report. GAO did, however, discuss the factual content of the report with EPA officials and has included their comments where appropriate. EPA officials generally agreed with the accuracy of the facts

but believed that, as presented, the report could be misread and suggested changes for presenting the facts. GAO made some revisions to the report on the basis of EPA's comments. GAO believes that the report is a fair and accurate presentation of the issues.

REFERENCE NO. 3

New York Times, March 22, 1994

Investigating a Medical Maze: Virus Transmission in Surgery

Hepatitis B was apparently spread by a doctor, but how?

By LAWRENCE K. ALTMAN, M.D.

In a cluster of cases that has mystified medical detectives, 18 patients who underwent heart surgery at two hospitals associated with the University of California at Los Angeles over a 10-month period in 1991 and 1992 developed hepatitis B several weeks after their operations.

The sleuths believe they know who the carrier of the virus was: a surgeon. But only after more than a year and a half of investigation do they have a clue as to how the patients became infected.

By reviewing medical records, interviewing doctors and patients and testing their blood, epidemiologist traced the cluster in July 1992 to a surgeon-in-training who had not been immunized against the viral liver infection. Over the 10 months, the surgeon, who has not been identified, had been involved in operations on 142 patients at both the U.C.L.A Medical Center and the Wadsworth Veterans Administration Hospital. Of these, 18, 13 percent developed hepatitis B infection. Of at least 155 patients operated on by other surgeons at the two hospitals, none developed hepatitis B.

A team of experts from the Federal Centers for Disease Control and Prevention in Atlanta, U.C.L.A. and the Los Angeles County Health Department tested as many of the 18 patients as they could, and found that in every one the virus was identical to that of the surgeon. The team reported its findings at a meeting in Atlanta last month.

The evidence was so compelling that the doctor stopped working as a surgeon as soon as the cluster was detected. He is now working in the operating room of an unidentified hospital and not performing surgical procedures, according to Dr. James Cherry, a U.C.L.A. hospital epidemiologist and an author of the report.

The doctor had presumably been infected by a patient, whom the detectives were not able to identify. It takes about six months from the time of exposure before symptoms, including fatigue and jaundice develop. In the doctor's case, this occurred about halfway through a period in which the patients were infected. The epidemiologist suspect he infected some patients while incubating the disease.

The doctor was tested, found to have hepatitis B and was permitted by the hospital to return to surgery after about two weeks, even though he was now a carrier of the virus.

The sleuths had solved one dimension of the outbreak. Still, a mystery remained: how did the surgeon transmit the virus to so many patients?

He did not recall having stuck himself with a needle or sharp instrument. If such a puncture tore his glove, a

few drops of virus-contaminated-blood could have leaked into a patient's body. This is also the way the surgeon himself might have become infected, though by the time he developed symptoms six months later, he had no recollection of any such incidents.

But as Dr. David M. Bell, an expert on hospital infections for the Centers for Disease Control and Prevention and a co-chairman of the meeting pointed out, the surgeon would have had to injure himself scores of times to infect so many patients, Colleagues had not noticed that the surgeon's operating-room techniques were flawed or sloppy.

After spending months pursuing many leads without conclusively identifying a specific mechanism for transmitting the virus, the epidemiologist decided to pick up on one of the surgeon's own suggestions.

"He mentioned that when he operates, on occasion his fingers would become irritated, and he speculated that perhaps the pressure of tying sutures might have something to do with the transmission" of the virus, said Dr. Rafael Harpaz, a CDC epidemiologist who headed the team, in an interview.

So the surgeon cooperated in a simulation of his surgical technique. In a sort of surgical marathon, he tied knots for an hour, far more than he would do in a real operation. When he finished the simulation, his fingers were bruised. The inside of each glove was washed, and the washings were sent to a laboratory. To the investigators' surprise, virus was found in the washings, offering a possible explanation of how the surgeon might have transmitted the virus to his patients: it might have

escaped through tiny holes in his surgical gloves, the result either of manufacturing imperfections or tears from use.

Surgeons often note minor bruising from suturing and the pressure of holding instruments, but they generally do not equate such irritations with the seriousness of an accidental glove puncture from a needle, sharp instrument or piece of bone.

It takes just a few drops of hepatitis B-contaminated blood to be infectious. Studies show that the risk of transmitting hepatitis B from a needle stick is about 100 times as great as the risk of transmitting the AIDS virus.

Transmission of hepatitis from an infected patient to a health care worker is not unusual. But Dr. Harpaz said the Los Angeles cluster is one of just seven reported worldwide since 1987 of transmission in the other direction, affecting some 44 patients altogether. Ability to transmit hepatitis B correlates strongly with the presence of the so-called e antigen in the blood. The antigen often remains but can disappear over time.

In several of the clusters, health officials were not able to determine how the hepatitis virus had been transmitted to a patient. They assumed that the doctor was sloppy and violated standard infection-control practices. But Dr. Bell of the CDC said that there was little scientific data to support this assumption and that "we need to re-examine it in light of the findings" from the Los Angeles cluster.

In none of the other cases were simulations and laboratory studies used to study how transmission might

have taken place. Dr. Harpaz said, while Dr. Bell and other experts cautioned against generalizing from the Los Angeles cluster until additional studies are done, he said that if the finding is confirmed, he hoped it could lead to improving gloves to help prevent transmission.

Although the spread of hepatitis B has never been linked to sweat, participants at the meeting suggested this might be a mode of transmission. If so, they speculated that contaminated sweat could leak through a hole in surgical gloves into a patient's body, thus posing a previously unrecognized risk.

Like HIV, the virus that causes AIDS, the hepatitis B virus is transmitted by blood and through sexual contact. But unlike HIV, hepatitis B can be prevented by a vaccine.

Dr. Craig Shapiro of the Centers for Disease Control, who is an author of the Los Angeles study, said that until recent years, an estimated 250 health care workers a year developed hepatitis B and eventually died from it. The figure now is about 100 a year, in part due to a recommendation in 1991 from the Federal Occupational Safety and Health Administration that surgeons and other health care workers be immunized to prevent hepatitis B infection.

One puzzle in the Los Angeles outbreaks is why the surgeon had refused the vaccine, which would have protected himself and his patients. Because health officials have not identified him, there is no way to ask him why he had not been vaccinated to protect himself and his patients. But Dr. Cherry of U.C.L.A. said the surgeon had been offered the vaccine at a hospital he had worked at before coming to U.C.L.A.

Dr. Quentin R. Stiles, a retired chest surgeon in Los Angeles who is a spokesman for the American College of Surgeons, said "A lot of surgeons are like that, mostly older ones," in declining to take the very immunization that they recommend to their patients. He characterized these older surgeons as "a very reluctant bunch" in changing their ways.

Because of their exposure to blood, surgeons run a particular occupational risk of getting hepatitis B. Yet the college has not conducted a survey to determine how many surgeons have taken the series of three hepatitis B shots. The vaccine has been available since 1981.

Dr. Stiles recently told a group of surgeons that they face a greater risk from hepatitis B than from HIV. When he asked how many in the audience had been immunized against hepatitis B, fewer than half said they had, and most of them were younger surgeons. Immunizing surgeons against hepatitis B "is the kind of thing we ought to be insisting upon," Dr. Stiles said.

In 1992, the Association of American Medical Colleges in Washington recommended that all medical students be immunized against hepatitis B before their first contact with patients and with blood products and human tissue. Immunization is of no benefit after infection with the virus. The association has not conducted a survey to determine compliance with the recommendation.

While the hospital's decision to allow the doctor to keep on operating even after he was found to be infected with the virus may seem unusual, it is in compliance with Federal guidelines. These call for local committees to

decide whether an infected surgeon can continue operating or performing procedures in which patients could be exposed to infected blood. In deciding, the committee evaluates a number of factors that might bear on the risk of transmission, like the skill of the worker and the kinds of procedures that the individual performs.

Health officials are aware that nothing less than the doctor's career is at stake in the decision.

The policy of the Los Angeles County Health Department, according to Dr. Shirley L. Fannin, an official of the department, is to allow infected doctors to continue working. But the doctor is informed that if one case of transmission is detected, limitations will be imposed on his practice.

REFERENCE NO. 4

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TRANSMISSION OF HEPATITIS B VIRUS TO MULTIPLE PATIENTS FROM A SURGEON WITHOUT EVIDENCE OF INADEQUATE INFECTION CONTROL

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Abstract *Background.* Although about 1 percent of surgeons are infected with hepatitis B virus (HBV), transmission from surgeons to patients is thought to be uncommon. In July 1992, a 47-year-old woman became ill with acute hepatitis B after undergoing a thymectomy in which a thoracic-surgery resident who had had acute hepatitis B six months earlier assisted.

Methods. To determine whether the surgeon transmitted HBV to this patient and others, we conducted chart review, interviews, and serologic testing of thoracic-surgery patients at the two hospitals where the surgeon worked from July 1991 to July 1992. Hepatitis B surface antigen (HBsAg) subtypes and DNA sequences from the surgeon and from infected patients were determined.

Results. Of 144 susceptible patients in whose surgery the infected surgeon participated, 19 had evidence of recent HBV infection (13 percent). One of the hospitals

was selected for additional study, and none of the 124 susceptible patients of the other thoracic surgeons at this hospital had evidence of recent HBV infection (relative risk, ∞; 95 percent confidence interval, 4.7 to ∞). No evidence was found for any common source of HBV other than the infected surgeon. The HBsAg subtype and the partial HBV DNA sequences from the surgeon were identical to those in the infected patients. Transmission of the infection was associated with cardiac transplantation (relative risk, 4.9; 95 percent confidence interval, 1.5 to 15.5) but not with other surgical procedures. The surgeon was positive for hepatitis B e antigen and had a high serum HBV DNA concentration (15 ng per milliliter). Our investigations identified no deficiencies in the surgeon's infection-control practices.

Conclusions. In this outbreak there was surgeon-to-patient HBV transmission despite apparent compliance with recommended infection-control practices. We could not identify any specific events that led to transmission. (N Engl J Med 1996;334:549-54). ©1996, Massachusetts Medical Society.

APPROXIMATELY 24 million operations are performed annually in U.S. hospitals by an estimated 133,000 surgeons.[1] The Centers for Disease Control and Prevention (CDC) estimate that 1900 U.S. surgeons are chronically infected with hepatitis B virus (HBV), but reports of surgeon-to-patient transmission of the virus are uncommon.[2] Transmission of HBV to patients has been associated with health care workers with highly infectious disease who were positive for hepatitis B e antigen (HBeAg) and has generally involved breaches in standard infection-control practices, although correction of these

deficiencies has not always prevented additional instances of transmission.[3-6] We report an outbreak of HBV infection associated with an HBV-infected thoracic-surgery resident and suggest potential mechanisms of transmission.

METHODS

In July 1992, a 47-year-old woman without identified risk factors became ill with acute hepatitis B four months after undergoing a thymectomy in which a thoracic-surgery resident participated. This surgeon was found to be susceptible to HBV on testing in December 1989 before completing a general-surgery residency elsewhere. He began the thoracic-surgery residency program in July 1991 after a year of research. He was offered the hepatitis B vaccine but never received it. In January 1992, he became fatigued, and in February he had jaundice with detectable hepatitis B surface antigen (HBsAg) and IgM antibody to hepatitis B core antigen (anti-HBc). He withdrew from surgical duty until March 1992, when his symptoms resolved, and he returned to practicing surgery having had no additional tests for HBsAg or HBeAg. He was still positive for HBsAg and HBeAg in July 1992, when the index patient was identified, and was relieved of surgical duties pending an investigation.

To determine whether other patients were infected with HBV, we obtained blood specimens in September 1992 from patients operated on by the surgeon during the study period, July 1991 to July 1992. The surgeon worked at two hospitals during this period, referred to here as Hospital A and Hospital B. For specimens collected

within six months after surgery, we asked susceptible patients to provide second specimens six months or more after surgery to permit the detection of later seroconversion. Chart reviews and interviews of patients or their parents were conducted with standardized forms; demographic data and information about surgical characteristics, prior HBV infection, and community risk factors for infection were recorded. Sexual and household contacts of infected patients were tested to exclude them as sources of transmission. Patients were defined as having acute HBV infection (case patients) if they were IgM anti-HBc-positive or if they were seronegative within the year before surgery or on initial testing and positive for HBsAg or anti-HBc on final testing.

Retrospective Cohort Studies

A retrospective cohort study was conducted at Hospital A to determine whether the surgeon had transmitted HBV to the index patient and possibly others. We compared the risks of infection among patients he operated on and among patients who underwent thoracic surgery without his participation from November 1991 to July 1992. Patients who had thoracic surgery without the surgeon's participation were contacted by letter and follow-up telephone call and asked to provide demographic information and serum for evaluation. This retrospective cohort study included all patients who underwent thoracic surgery during the months when the surgeon was not working at Hospital A and every third patient of other thoracic surgeons during the months when he was working there.

A second retrospective cohort study evaluated risk factors for infection among the patients the surgeon operated on at Hospital A, where data were more complete. The study period was the same as that of the first retrospective cohort study. Data regarding characteristics of operations and surgical procedures were collected with standardized forms.

Additional information was collected by informally interviewing the surgeon, operating-room personnel (thoracic-surgery fellows, attending physicians, anesthesiologists, scrub nurses, and perfusionists), and nurses in the intensive care unit. The work schedules of nurses, phlebotomists, and respiratory therapists were reviewed for possible opportunities for transmission by circulating hospital personnel. The vaccination records and the results of serologic tests for HBV of operating-room personnel were reviewed, as were records of transfusions in patients. Testing was conducted at the CDC for HBsAg and antibody to HBsAg (anti-HBs) by radioimmunoassay and for anti-HBc and IgM anti-HBc by enzyme immunoassay (Abbott Laboratories, North Chicago, Ill.). In several instances, samples were tested at local clinical laboratories. Samples with detectable HBsAg were analyzed for HBsAg subtype by enzyme immunoassay with monoclonal antibodies.[7]

Analysis of Serum Samples

Serum from the surgeon, from the infected patients, and from an unrelated, acutely and chronically infected convenience sample of controls from the state in which the outbreak occurred were subjected to amplification by

the polymerase chain reaction (PCR) to detect HBV DNA. Twenty microliters of serum was digested with proteinase K solution for one hour at 65°C, followed by phenol-chloroform extraction and alcohol precipitation. HBV1858 (5'ACTGTTCA-AGCCTCCAAGCTG3'), HBV2437 (5'TTGAGATCTTCTGCGACGCGGC3'), and 5 units of Taq polymerase were added to the precipitate for amplification (30 cycles consisting of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 45 seconds). The samples were purified and the sequence of 160 bases in the core region was determined with use of HBV1858P[8] or the ABI automated sequencer and dye terminators (Applied Biosystems, Foster City, Calif.). The sequences were analyzed with the Pileup program, which performs progressive, pairwise comparisons and plots the results in a dendogram to indicate similarity of sequences.[9]

The HBV DNA concentration in the surgeon's serum was determined by dot blot hybridization[10] and by PCR end-point dilution. Ten-fold serum dilutions were amplified by PCR and evaluated by agarosegel electrophoresis. The threshold of HBV DNA detectability was compared with that of similarly diluted serum containing 100 million chimpanzee-infectious particles per milliliter.

Statistical Analysis

The relative risks and 95 percent confidence intervals were calculated with the use of Epi Info[11]; the associations between exposures and infection were assessed by univariate and stratified analysis; and the significance of differences in proportions was assessed by the chi-square

test with the Mantel-Haenszel correction for independent samples or with Fisher's exact test.

RESULTS

Identification of Cases

The surgeon operated on 239 patients (162 at Hospital A and 77 at Hospital B) from July 1, 1991, through July 16, 1992. Twenty-eight patients died before the investigation; none had recognized evidence of hepatitis. Of the remaining 211 patients, 184 (87 percent) were available for initial serologic testing, with 170 (81 percent) tested six months or more after surgery. Of these 170, 11 reported prior HBV infection or hepatitis B vaccination and had markers consistent with their histories; they were excluded from the analysis. Nineteen patients had evidence of acute HBV infection, as indicated by the presence of IgM anti-HBc or by anti-HBc seroconversion. Fifteen additional patients had HBV serologic markers but were negative for IgM anti-HBc and had no evidence indicating the presence or absence of seroconversion; seven of these patients had other risk factors for HBV infection. For purposes of analysis, these 15 patients were assumed to have been infected before surgery. The overall attack rate was therefore 13 percent (19 of 144) among susceptible patients available for follow-up.

The 19 case patients with acute HBV infection ranged in age from 14 months to 83 years (median, 51 years). Six (32 percent) had symptoms of acute hepatitis, one of whom required hospitalization. Three of the remaining patients died of other causes. Chronic HBV infection developed in 9 of the 16 surviving case patients (56

percent); 3 of these 9 had been receiving immunosuppressive therapy, and another was two years of age.

All 19 case patients or their parents reported no other risk factors for HBV infection. Sexual and household contacts of all but three patients underwent HBV testing; none were HBsAg-positive. Of the 19 case patients, 15 had had surgery at Hospital A and 4 at Hospital B. The procedures included coronary-artery bypass surgery (eight), orthotopic heart transplantation (four), repair of congenital heart defects (four), valve replacement (one), thymectomy (one), and open-lung biopsy (one). The procedures occurred throughout the study period without apparent clustering in time (Fig. 1), even after we controlled for the number of susceptible patients undergoing surgery each month (data not shown).

Determining the Source of the Outbreak

We conducted a retrospective cohort study to determine whether patients of other surgeons at Hospital A had acute HBV infection: a sample of 280 of 510 such patients who underwent surgery from November 1991 through June 1992 was selected for evaluation, including all patients who underwent thoracic surgery at Hospital A while the HBV-infected surgeon was not working there and every third patient of other thoracic surgeons at Hospital A while he was working there. Of these patients, 259 were alive; 124 consented to be tested, were determined to be susceptible at the time of surgery, and had six-month follow-up data available (Table 1). None of the 124 had evidence of recent HBV infection. This result contrasts with the 15 who had such evidence (14 percent)

among the 106 patients who were operated on by the surgeon at Hospital A (Table 1) (relative risk, ∞; 95 percent confidence interval, 4.7 to ∞).

The two cohorts did not differ significantly according to age, sex, race, or the distribution of procedures (data not shown), although the average duration of surgery was longer for patients operated on by the infected surgeon than for patients operated on by other surgeons (5.4 vs. 4.5 hours; $P<0.001$). When patients undergoing thoracic surgery who had evidence of prior, but not recent, infection were included in the analysis as case patients, the difference in infection rates for the two cohorts remained significant: 24 of the 115 patients operated on by the infected surgeon were HBV-infected (21 percent), in contrast with 7 of the 131 patients operated on by other surgeons (5.3 percent) (relative risk, 3.9; 95 percent confidence interval, 1.8 to 8.7).

Opportunities for transmission from other nosocomial exposures were investigated. Twelve of 15 case patients at Hospital A (80 percent) had received blood transfusions, all from different donors. No other surgeon, anesthesiologist, nurse, phlebotomist, or respiratory therapist had documentation of HBV infection, and none treated more than seven case patients. Transmission to patients occurred at both Hospital A and Hospital B; the surgeon was the only common factor at the two hospitals.

The HBsAg subtype from the surgeon and from 13 of the 19 case patients at both hospitals for whom subtyping could be performed was adw2. We amplified HBV DNA from the surgeon, 9 case patients, and 19 unrelated community controls (7 with acute infection and 12 with

chronic infection). The sequences from the surgeon and the case patients were identical. The sequences from all but 4 of the 19 community controls were different from each other and from the sequence from the surgeon (Fig. 2).

Evaluation of Surgery-Related Risk Factors for HBV Infection

To identify risk factors for HBV infection, we conducted a retrospective cohort analysis of the patients the surgeon operated on at Hospital A (Table 2). The infection rates did not differ according to sex or age. The rate was higher among whites than in other racial groups. The infection rate was higher among patients who received blood products during surgery or who had surgery lasting 5.5 hours or longer, but these differences were not statistically significant. The surgeon's patients underwent a variety of surgical procedures, but the infection rate was increased only among patients who underwent cardiac transplantation (relative risk, 4.9; 95 percent confidence interval, 1.5 to 15.5). This association remained significant when the duration of the procedure and the use of blood products were controlled for (data not shown). The infection rates were not associated with emergency (as opposed to elective) surgery, the use of a perfusion pump or cell saver, the specific operating room, or prior sternotomy. The analysis of these associations was unchanged by the inclusion of the 15 patients with serologic markers of HBV infection but without evidence of recent seroconversion (data not shown).

Additional Fact-Finding

The surgeon reported no risk factors for HBV infection and was unaware of any percutaneous exposure to blood from HBV-infected patients. He indistinctly recalled only one or two needle sticks during the period under investigation, and he reported no injuries from sternal wires or other sharp objects. He reported that he always handled sharp objects with an instrument and did not blindly palpate suture needles. Although the surgeon reportedly often applied hemostatic material to sternal incisions with his gloved hands rather than with the protection of a sponge (the usual practice of other surgeons at the two hospitals), he recalled no glove punctures from this procedure. He performed no invasive procedures on case patients in the intensive care unit or the recovery room.

Other surgical personnel attested to the surgeon's good technique. He was left-handed, which sometimes interfered with the passing of instruments or simultaneous suturing by more than one surgeon. He did not use double gloves, but after contracting hepatitis B he modified his behavior by frequently changing gloves during operations. All surgical staff members, including the surgeon, reported that blood was routinely present on their hands when they removed their gloves after an operation, whether or not visible tears were present in the gloves and regardless of the type of gloves used.

In previous years, the surgeon had had a skin irritation that resolved after he changed to the routine use of hypoallergenic latex gloves. In addition, he had periodic

pain over the radial side of the index fingers that he attributed to shear forces from tying sutures.

The serum HBV DNA concentration in the surgeon just after the index patient was identified was 15 ng per milliliter. The serum was estimated by semiquantitative PCR to contain 1 billion infectious particles per milliliter.

Discussion

The infected thoracic surgeon whom we studied transmitted HBV to at least 19 patients during surgery. No patients undergoing procedures performed by other thoracic surgeons had evidence of recent infection. The timing of the infections and the absence of other identified sources of infection among the case patients were consistent with transmission from the surgeon during surgery. The infections occurred at two different hospitals without common equipment or staff members other than the surgeon. The presence of HBsAg subtype adw2 in the surgeon and in the 13 cases patients in whom the subtype of the antigen could be determined is unlikely to have occurred by chance alone.[12] The DNA sequences of the HBV core region from the surgeon and from all 9 case patients who could be evaluated were identical and were different from that of all but 4 of the 19 community isolates.

Although reporting of HBV infection is not complete, both outbreaks and sporadic transmission of HBV from surgeon to patient appear to be uncommon. Evidence that the risk is low is limited and includes retrospective studies involving patients of infected health care workers,[13-16] two case-control studies of patients with

acute hepatitis B that found no association between disease and surgical history[17] (and unpublished data), and the relatively small number of reported outbreaks of HBV given the estimated pool of infected surgeons.

Outbreaks provide information about specific mechanisms of transmission of HBV from surgeon to patient. Since the early 1970s, 29 such clusters have been reported worldwide,[3,5,18-24] including 9 involving thoracic surgeons.[5,19,22-24] Data from these outbreaks indicate an increased risk of HBV transmission from HBeAg-positive surgeons and during particularly invasive procedures.[5,21,25] Transmission during many of these outbreaks was presumed to be caused by deficiencies in infection-control measures. Although this outbreak involved a high attack rate, our investigation did not identify any breaches in infection-control practices, despite an extensive search for potential modes of transmission. Unreported or unrecalled percutaneous exposures by the surgeon or operating-room staff are unlikely to explain such a high rate of transmission.

Although this is the first reported outbreak involving a thoracic surgeon in the United States, four such outbreaks have been reported in the United Kingdom during the past decade.[5,24] We found no specific features characteristic of thoracic surgery that were associated with transmission. Surgical fields are generally well visualized during thoracic surgery, and blind needle palpation is not often practiced. Thoracic surgery is, however, inherently highly invasive and of long duration, and these features have been linked to percutaneous exposure,[26-28] glove failure,[29-31] and HBV transmission.[21] Indeed, whether caused by the duration of surgery or by specific factors

such as the closure of sternotomy incisions, frequent glove punctures during thoracic surgery have been reported.[26,28,31,32] In this outbreak, there was no association of HBV infection with the duration of surgery or the use of blood products (a possible indication of the invasiveness of a procedure); two case patients, in fact, underwent brief procedures requiring no blood products (a thymectomy and an open-lung biopsy). We found no associations between HBV transmission and specific procedures, with the exception of cardiac transplantation, although in relative terms these were not long or complex procedures. Perhaps the minimal infectious inoculum of HBV is lower for patients receiving immunosuppressive therapy. Some surgeons have suggested that closure of the median sternotomy incision is associated with injury, although data to support this assertion are inconclusive.[31,32] In our study, the surgeon's technique of applying hemostatic material to the sternal incision without a sponge may have caused injuries that were not apparent. However, one case patient underwent an open-lung biopsy that did not involve a median sternotomy.

This outbreak may have been related more closely to factors unique to the surgeon than to factors inherent in thoracic surgery: indeed, lung biopsy is a procedure with little resemblance to most other thoracic surgical procedures. Although HBeAg-positive persons almost always have highly infectious disease, the surgeon had an especially high concentration of HBV DNA during the outbreak, which may have contributed to aq high risk of transmission. The surgeon's technical skills were apparently not a factor, since operating-room personnel did not

recall that he had frequent needle sticks. The hand irritation experienced by the surgeon in previous years had resolved with the use of hypoallergenic latex gloves, and there was no evidence that the surgeon had dermatitis during the outbreak. Hypoallergenic gloves are subject to the same quality standards as standard surgical gloves.

The surgeon had pain over his index fingers during prolonged suturing. Other surgeons have described similar experiences to us; we are unaware of any studies addressing this phenomenon. While participating in a one-hour simulation of suture tying,[33] the surgeon acquired paper-cut-like lesions on his fingers, and HgsAg and HBV DNA were isolated from washings of his hands. Such lesions, combined with the failure of his gloves, may have allowed contamination of patients with HBV. Although gloves frequently have leaks during surgery,[27,28,31,34,35] they nonetheless appear to be fairly effective barriers against certain infections, even when leaks are present.[36] Although there is increasing evidence that double gloves can prevent exposure of surgeons to blood during surgery,[35] there is no evidence regarding the effectiveness of double gloves in protecting patients from blood-borne infections. Furthermore, advisory groups and professional organizations have not generally recommended the use of double gloves by surgeons. Additional studies are needed to assess the validity and generalizability of the suture-tying simulation and to define the role of gloves in preventing the intraoperative transmission of HBV.

This outbreak has had tragic consequences for the case patients, their families, and the surgeon, who has left surgical practice indefinitely. The entire episode could

have been prevented had the surgeon received the hepatitis B vaccine.

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REFERENCE NO. 5

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Efficiency of 42 brands of face masks and 2 face shields in preventing inhalation of airborne debris

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Use of face masks by dental clinicians has increased as a result of the AIDS epidemic.[1] In response to clinicians' concerns about infectious disease transmission, the American Dental Association (ADA) and Centers for Disease Control (CDC) issued recommendations for use of preventive measures, including face masks, during dental treatment.[2,3] The use of face masks is now required by the Occupational Safety and Health Administration (OSHA).[4,5] However, none of these agencies provided information on relative merits of different types of facial barriers, which might give one the impression that all facial barriers are equally effective. While this may be true for blockage of large particles and splatter, dentists also must protect themselves from aerosols that are generated during dental procedures and remain airborne following patient treatment.[6-11]

In clinical studies characterizing aerosols by particle size, Pollok et al. showed that about half of such particles were 3 µm or smaller.[6] Aerosol particles of this size have been shown to be particularly critical. In studies of 26 nonsmokers, Chan et al. demonstrated that particles about 3µm in size have maximum deposition in the alveoli.[12] According to Day et al., this deep penetration

into the respiratory tract increases the infectivity of microorganisms.[13] Hence, face masks for dental use should block particles down to at least 3 μm in size.

Studies of toxicological warfare have confirmed that airborne organisms can cause infections in man.[14-15] To determine whether tooth-cutting procedures could lead to aerosols containing oral pathogens in numbers sufficient to infect humans, Belting et al. cut cavity preparations on tuberculosis patients with positive sputum and cultured air samples following treatment.[8] They showed conclusively that oral tuberculosis organisms become airborne in numbers sufficient to infect clinicians. At present, the use of high-filtration facial barriers with close peripheral fit is the best known way to minimize inhalation and infections from this type of airborne bacteria.

Clinicians need to know the differences among the various brands of facial barriers on the market. This study compares the efficacy of various face masks and shields in blocking airborne debris.

Materials and methods

The test protocol specified four evaluations:

(1) Three samples of all face masks studied were tested in vitro.

(2) Selected face masks worn by clinicians were tested to determine clinical effectiveness.

(3) Selected face masks were worn in high humidity, then tested to determine the high humidity's effect.

(4) Finally, a dye tracer test was performed on selected face masks and shields to determine importance of peripheral fit.

Collection of masks and shields

Brand names of face masks sold to dental clinicians were identified, and identical masks sold under different brand names were eliminated. However, when confidentiality agreements prohibited identification of manufacturers, similar masks were tested, as clinicians at times have no way to confirm manufacturers and often must identify face masks by brand name only. Face shields were selected on the basis of market popularity and amount of facial coverage. The table lists brand names of 42 face masks and 2 face shields evaluated.

Bacterial filtration efficiency testing

Evaluation of 42 brands of face masks – Three unused samples of each of 42 brands of face masks were coded and sent for bacterial filtration efficiency (BFE) testing at a commercial laboratory that routinely performs this standardized procedure developed by Nicholes (Fig.1).[16] A 24-hour culture of *Staphylococcus aureus* clinical isolate, strain Utah 15, at a concentration of approximately 10^4 colony forming units (cfu) per milliliter, was used in the following manner:

(1) The culture suspension was nebulized (Chicago nebulizer*) to create a cloud with a mean particle size of 3.0 μm.

(2) The cloud was drawn through the test mask at a rate of 1 ft^3/min. and onto a 6-stage

sampler (Andersen sampler†) where particles were separated by size onto one of 6 Petri plates containing soybean casein digest agar (Fig. 2a and 2b).

(3) The plates were incubated at 37°C for 48 hours; colony forming units were counted and probable hit values were computed according to Andersen's method.[17]

To determine the consistency of delivery for the challenge aerosol, control values were established through sampling without a filter. Acceptable limits for this aerosol density were between 1,700 and 2,700 cfu/ft.^{3} To determine proper functioning of the test system, a reference filter with a known BFE was tested. Challenge aerosol sampling and reference material evaluation were conducted for every 8 to 10 test samples.

The following formula was used to calculate filtration efficiency of all test masks:

$$\text{Percent BFE} = \frac{C - F}{C} \times 100,$$

where C (control) represents the number of particles sampled when no mask was used an F (filtered) represents the number of bacterial particles that passed through the test mask. Mean BFE percentages were computed for each set of three identical masks. One-way analysis of variance (AOV) and the Bonferroni multiple comparison procedure were used to determine differences in filtration efficiency among brands of masks.

Evaluation of four face masks after clinical use – The Aseptex mask,‡ Dental Surgical mask#, and Magic Arch#

masks were tested. Two general dentists and their primary chairside assistant wore coded face masks during patient treatment for one, two, three, and seven hours. Test periods were assigned randomly and repeated about eight times. No attempt was made to alter patient treatment. After each test, the mask was removed without contacting its surface, and BFE testing was performed. Mean BFE percentages were computed for each interval. One-way AOV and the Bonferroni multiple comparison procedure (alpha = 0.05) were performed to determine differences among intervals during which masks were worn, and between brands of masks tested and persons wearing the masks.

Performance of 6 face masks in humidity > 90 percent – ADDS-Air-Flow laboratory mask,** ADDS-Vanced laboratory mask**, Aseptex mask, Aseptex Plus mask, Duckbill surgical mask,‡ and the Dental surgical mask# were tested. Two subjects (a woman 54 kg and a man 100 kg) wore each of the test masks for 30 minutes in a chamber (42 inches x 24 inches x 24 inches). Their heads and shoulders were positioned inside the chamber, 6 and 12 inches, respectively, from an air-slurry polisher (Prophy Jet 30‡‡) operated on medium water and powder settings with an air pressure of 80 psi. After 30 minutes, the masks were removed without contacting their surfaces and BFE testing was performed as described above. BFE percentages were computed and plotted against mask type.

Dye tracer testing – The Aseptex mask, Magic Arch mask, Cover shield,## and Op-d-Op shield*** were tested. A test also was performed using the Aseptex mask in combination with the Op-d-op shield.

A subject's head and shoulders were positioned inside a chamber (30 inches x 30 inches x 36 inches) with a nebulizer (Micro Mist Aerosolizer #P220†††) emitting a dye (Blue Crayola Tempera‡‡‡) diluted with three parts Crayola Tempera‡‡‡ deionized water. Mean particle size of the airborne dye was 4.8 µm. The distance between the facial barrier and orifice of the nebulizer was 6 inches (Fig. 3).

Figures 4a to 4i illustrate the steps in the test procedures. A 2 inch x 2 inch cotton-filled gauze sponge (Healthco No. HCOC535051###) opened at its center was positioned so the nose was covered (Fig. 4g). Impermeable plastic was placed such that, for each facial barrier tested, inhaled air was allowed to enter:

(1) through the mask and peripheral border (Fig. 4c);

(2) through the mask material only (Fig. 4g); and

(3) through the peripheral border only (Fig. 4h). Figure 4i shows the results of a positive control test in which no facial barrier was worn. All tests were conducted for 10 minutes.

The gauze nose wraps from all but the combination Aseptex Mask/Op-d-op Shield tested were ranked according to area and intensity of dye stain by 21 evaluators who were not associated with the study. Kendall's coefficient of concordance was computed to determine agreement between evaluators.

Results

Bacterial filtration efficiency testing – In Figure 5, the 42 face masks tested are ranked in order of highest to lowest mean BFE scores. Lines connecting brand names indicate statistically non-significant scores, and overlapping lines indicate statistically similar groups. The test masks separated into two non-overlapping significance groups. The high filtration group included 22 face masks with mean BFE scores of 74 to 98 percent. All but two masks in this group were soft, pleated, rectangular masks. Two rigid, preformed, cup-style masks with a unique design (the ADDS-Air Flow laboratory and ADDS-Vanced laboratory masks) had filtration values of 91 to 92 percent. The scores of the 20 remaining masks were significantly lower (13 to 51 percent). In fact, all brands of conventional, cup-style face masks, including the Aseptex and Aseptex Plus brands, were in the low filtration group.

Figure 6 shows mean BFE percentages for 4 face masks worn during clinical treatment. The two soft, pleated, rectangular masks (Dental Surgical and Magic Arch) had efficiency scores of 97 to 99 percent, regardless of length of time worn. The Aseptex Plus proved to be statistically more efficient than the Aseptex. No statistical differences were observed with respect to persons wearing the mask.

Figure 7 shows the mean BFE percentages for 6 face masks tested under normal (about 50 percent humidity) and humid conditions (> 90 percent humidity); comparison of BFE percentages shows mask filtration was not

affected adversely by high humidity. The size and gender of the subject also had no effect on BFE scores.

Dye tracer testing – Figure 8 shows the gauze sponges worn over the test subject's nostrils during 10-minute dye tracer tests. Kendall's coefficient of concordance (0.993) showed high agreement between the 21 evaluators who ranked the sponges. In Figures 8a to 8i, the gauze sponges are shown as ranked, from least to most effective, by the evaluators. Results indicate the following:

(1) All test barriers blocked some airborne dye and proved more effective than no barrier (compare control gauze in Fig. 8a with all others).

(2) The face shields were least effective in blocking airborne dye (compare Fig. 8b and 8c with all others).

(3) The high-filtration mask provided the best defense against penetration (compare Fig. 8g, 8h, and 8i with all others).

(4) High-filtration material and close peripheral fit were needed for optimum blockage of aerosols (compare Fig. 8i with all others).

Figures 9a and 9b show leakage of dye tracer onto the interior surface of a high-filtration mask after 10 and 30 minutes of use. This problem was evident to varying degrees in all test masks. The colored dye enabled us to observe a problem not clinically apparent, because most dental aerosols (such as saliva and water) are colorless. Mask penetration is highly significant because it can bring airborne organisms into contact with the wearer's lips, nostrils, and skin.

In an additional test in which the Aseptex mask was used in combination with an Op-d-op Shield, aerosol penetration was diminished only slightly (Fig. 10). The combination did not provide the level of protection offered by the high-filtration face mask (Fig. 8g to 8i).

Discussion

Several theories concerning face masks were disproven in this series of tests. For example, filtration efficiency was not decreased by use in a humid environment for up to 30 minutes or by clinical use up to 7 continuous hours. Filtration efficiency was not affected by gender or size of the clinician. In addition, this study showed clearly that the test masks were not equally effective in preventing penetration of airborne debris. Shields proved to be substantially inferior to masks, owing to their lack of peripheral fit, which was shown to be as important as filtration efficiency in blocking airborne debris.

Size of airborne particles is important regarding the effectiveness of face masks. Investigators have demonstrated that dental procedures generate large quantities of aerosols 3 μm and smaller.[6] Such particles remain suspended at the end of treatment,[6,7] and can penetrate to the alveoli of the lower respiratory tract,[12] where their infectivity is greatly increased.[13] Therefore, dentists and staff should use facial barriers that block particles of this size, but our results showed only about half the test masks met this criterion. Conventional, preformed, cup-style face masks had low filtration. When choosing masks, dentists must consider the fact that 50 percent or more of deleterious airborne particles can filter through

this type of mask. After reviewing these data, two face mask manufacturers redesigned their preformed, cup-style face masks to achieve high BFE ratings (Triple Layer by 3M and Surgical Comfort by Healthco). These masks are now available.

It is clinically relevant to establish the length of time a face mask can be worn. This investigation showed neither high humidity nor use over time affected significantly the filtration of the masks tested. Dye tracer tests indicated the critical factor is soak-through of airborne debris. This process, called wicking, poses a threat when the wearer's lips and nostrils come in contact with moist areas, thus establishing direct mucous membrane contact with organism-laden material. Current mask design does not allow clinicians to determine when wicking has occurred, because interior surfaces of masks are not stained visibly when wet by colorless fluids. Given present mask technology, we agree with Craig et al., who suggested mask changes after 20 minutes in aerosol and 60 minutes in nonaerosol environments.[18] These recommendations were based on culturing of masks worn clinically. When worn longer than the recommended times, the masks became impregnated with microorganisms and, thus, were a source of, rather than a barrier to, potential pathogens.

When gathering information on filtration efficiency of face masks, dentists need to know that efficiency ratings can be generated by two tests. One is the BFE procedure performed here; the procedure is well suited to dental use of face masks. The other is the Greene and Vesley test,[19] which measures filtration from the inside of the mask outward through use of large particles. This test

is relevant to surgeons whose primary concern is deposition of droplets from the operator onto the patient. Unfortunately, Greene and Vesley test percentages often are used to promote masks to dental personnel because such ratings are substantially higher due to use of larger-sized test particles.

Filtration efficiency ratings of preformed cup-style masks increased slightly after three hours of clinical use. This phenomenon is common in BFE testing of cup-style masks, and is thought to be caused by impingement of droplets onto the mask. Some pores are blocked, which temporarily increases filtration efficiency. Droplets then coalesce, reducing filtration efficiency.

Confidentiality agreements between manufacturers and distributors often result in very nearly the same product being sold in different packaging. In this study, testing of some similar masks was performed because, in some cases, positive identification of manufacturers could not be obtained. Testing was justified on the premise that data was needed for specific brand names for which manufacturers could not be identified positively. However, test results indicate similarity of manufacturing technology, rather than the manufacturer, was the key factor in mask filtration efficiency. Test masks were separated by BFE ratings into two distinct groups, although masks from more than two manufacturers were evaluated. With only a few expectations, the 42 masks were divided into a soft, pleated, rectangular, high-filtration group; and a rigid, preformed, cup-style, low-filtration group.

Conclusions

Bacterial filtration efficiency ratings here indicate 22 of the 42 brands of test masks ensure efficient blockage of airborne debris. Protective barriers against small-particle aerosols should be selected from among this group (Fig. 5). Preformed, cup-style face mask with a conventional design, and face shields all provided significantly less blockage of airborne particles.

The main factor in deciding when to discard a face mask of nay kind was degree of wicking. Because penetration of fluids such as water and saliva cannot be observed on the mask's interior surface, the recommendations of Craig et al. (to change masks after 20 minutes in aerosol and 60 minutes in nonaerosol environments) should be followed.[18] They showed that masks worn for longer periods became a source of infectious material.[18]

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**Dependable Scientific Glass Co., Salt Lake City, UT 84115.*

†Andersen 2000 Inc., Peachtree City, GA 30269.

‡3M Co., Dental Products Division, St. Paul, MN 55144.

#Alpha Pro Tech, Inc., North Salt Lake City, UT 84054

***American Diversified Dental Systems, Anaheim, CA 92806.*

††Baxter, Deerfield, IL 60015.

‡‡Dentsply, York, PA 17405.

##GRD Products, Grand Junction, CO 81501.

****Op-d-Op Inc., Roseville, CA 95678.*

†††Micro Mist Corp., Hudson, OH 44236.

‡‡‡Binney and Smith Inc., Easton, PA 18044.

###Healthco Inc., Boston, MA 02116.

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Table 1. Alphabetical list by brand name of 42 face masks and 2 face shields

Brand name	Company	Description
(Face masks)		
1. 8500 Non-Toxic particle mask	Colwell	Rigid, preformed cup, 1 elastic strap
2. ADDS-Air Flow laboratory mask	American Diversified Dental Systems	Rigid, preformed cup, 2 elastic straps
3. ADDS-Vanced laboratory mask	American Diversified Dental Systems	Rigid, preformed cup, 2 elastic straps
4. Asept-All Com-Fit mask	Sultan Chemists	Rigid, preformed cup, 1 elastic strap
5. Aseptex mask	3M	Rigid, preformed cup, 1 elastic strap
6. Aseptex Plus mask	3M	Rigid, preformed cup, 1 elastic strap
7. Classic surgical mask	Baxter	Soft, pleated rectangle, 4 ties
8. Cone Classic surgical mask	Baxter	Rigid, preformed cup, 1 elastic strap
9. Contour medical mask	Island Poly	Rigid, preformed cup, 1 elastic strap
10. D-Sep mask	Barrier Protection	Soft, pleated rectangle, 1 elastic thread
11. Dental procedure mask	Alpha Dental mask	Soft, pleated rectangle, 1 elastic strap
12. Dental procedure mask	Healthco	Soft, pleated rectangle, 2 elastic ear loops
13. Dental surgical mask	Alpha Dental	Soft, pleated rectangle, 1 elastic strap
14. Disposable blue mask	Biotrol	Rigid, preformed cup, 1 elastic strap
15. Duckbill surgical mask	Baxter	Soft, duckbill rectangle, 4 ties
16. Dust & respirator mask	Biosafety Systems	Rigid, preformed cup, 1 elastic strap
17. Face mask frame with disposable insert	Biotrol	Rigid, preformed cup insert, 1 elastic strap over nose
18. Facelock wet mask	Alpha Dental	Soft, pleated rectangle, 1 elastic strap
19. Fluid guard surgical mask	Healthco	Soft, pleated rectangle, 2 elastic ear loops
20. Fluid shield surgical mask	Baxter	Soft, pleated rectangle, 4 ties
21. Fog-free surgical mask	Baxter	Soft, pleated rectangle, 4 ties
22. High-filtration isolation mask	Baxter	Soft, pleated rectangle, 2 elastic ear loops
23. Magic arch mask	Alpha Dental	Soft, pleated rectangle, 1 elastic strap
24. Molded mask	Kent Dental	Rigid, preformed cup, 1 elastic strap
25. Molded regular mask	Schein	Rigid, preformed cup, 1 elastic strap
26. Molded surgical mask	Professional Discount Mart	Rigid, preformed cup, 1 elastic strap
27. Pleated mask	Professional Discount Mart	Soft, pleated rectangle, 4 ties
28. Pleated form fit mask with ear loops	Kent Dental	Soft, pleated rectangle, 2 elastic ear loops
29. Polyfil air mask	Kent Dental	Plastic foam strip, 2.5 inch diameter holes serve as ear loops
30. Preformed cup mask with fluid guard	Cottrell	Rigid, preformed cup, 1 elastic strap
31. Protec-Shield mask	Alliance	Rigid, preformed cup, 1 elastic strap
32. Protec-Shield flat mask	Alliance	Soft, pleated rectangle, 2 elastic ear loops
33. Shield-pleated ear-loop mask	Cottrell	Soft, pleated rectangle, 2 elastic ear loops
34. Shieldmate mask	Cottrell	Soft, pleated rectangle with clear plastic eye covering attached, 4 ties
35. Sigma designer mask	Endicott & Beaman	Soft, pleated rectangle, 4 ties
36. Soft, ear-loop mask	Biosafety Systems	Soft, pleated rectangle, 2 elastic ear loops
37. Surgical antifog mask	Healthco	Soft, pleated rectangle, 2 elastic ear loops
38. Surgical mask	Healthco	Rigid, preformed cup, 1 elastic strap
39. Surgine II mask	Johnson & Johnson	Soft, pleated rectangle, 4 ties
40. Surgine II antifog mask	Johnson & Johnson	Soft, pleated rectangle, 4 ties
41. Tie-on surgical mask #1818	3M	Soft, pleated rectangle, 4 ties
42. Ulti-mask	Baxter	Soft, pleated rectangle, 4 ties
(Face shields)		
1. Cover shield	Glen R. Dean	Clear, rigid, plastic; molded to fit plastic glasses frame and facial contours
2. Op-d-op shield	Healthco	Clear, rigid, plastic attached to plastic head visor

REFERENCE NO. 6

VOLUME 19, ISSUE 9 – SEPTEMBER 1995
CRA Newsletter

SUBJECT: OPERATING GLOVES, IMPORTANT NEW TRENDS

Never before have so many *dental* clinicians used operating gloves routinely. Extensive use has revealed sensitivities not anticipated. *Glove manufacturers are now seeking alternatives because glove sensitivity can force clinicians to cease practice.* This report on new *powder free* alternatives focuses on: (1) Quality, clinical characteristics, & cost of 32 brands of *powder free* gloves; & (2) Clinical & lab data that strongly implicate glove defects in virus transfer.

METHODS:

A. GLOVE ACQUISITION – 6 boxes of about 100 gloves each from 32 powder free glove brands were acquired from 20 sources in 12 U.S. states from January through March '95.

B. CLINICAL CHARACTERISTICS TEST – 300 each of the 32 glove brands (9600 total) were used during routine clinical treatment by dentists & auxiliaries at 29 clinical sites in 19 U.S. states & rated according to: (1) Fit, (2) Tactile sensitivity, (3) Taste, (4) Tackiness when wet, (5) Resistance to tearing, & (6) Cuff length (adequate to pull *over* long sleeve).

C. MANUFACTURING DEFECTS TESTS – About 290 each of the 32 glove brands (9280 total) were tested *in-vitro* for perforations (FDA Water Leak Test) & 9 of each brand (288 total) were tested for time before hydration occurred (Dental-Alert Monitor by Novatec [713] 266-1976).

1. GLOVE QUALITY (defects & hydration time), CLINICAL CHARACTERISTICS, & COST OF 32 POWDER FREE GLOVES.

Gloves are ranked best to worst by *combination* of low glove defects & hydration times reaching 30 minutes.

GLOVE NAME, ORDER NUMBER & (SIZES)	GLOVE DEFECTS	HYDRATION TIME	CLINICAL RATING*	RETAIL COST /GLOVE/CASE
Biogel-D, 100-(51/2-9) (COTROL)	0.7%	28 min.	5.2	62¢ CONTROL
Crosstex Powderless, CTPLG-(S-M-L)	1.0%	29 min.	4.7	10¢
Ansell Conform, 114-(S-M-L)	2.4%	30 min.	4.6	11¢
Golden Glove, (no order #)-(S-M-L)	2.4%	30 min.	5.1	8¢
Aladan Tri-Clean 110, 0225240-(S-M-L)	3.8%	30 min.	4.2	12¢
Microflex Diamond Grip, G-4013-(S-M-L)	1.4%	22 min.	5.2	9¢
Tronex P.F., 3010-(XS-S-M-L-XL)	1.4%	22 min.	4.5	9¢
QualiTouch Powder Free, 40-95-(XS-S-M-L)	2.8%	26 min.	4.9	11¢
Royal Shield, (no order #)-(XS-S-M-L)	4.4%	30 min.	5.1	6¢
Latex Medical Exam Powder Free,100-(S-M-L)	4.6%	30 min.	4.0	10¢
Pharmaseal Flexam, 8878-(S-M-L)	4.6%	30 min.	4.4	7¢
Alpine Powder Free, (no order #)-(S-M-L)	1.4%	20 min.	4.6	9¢
Tillotson Powder Free Plus, (02-450-(S-M-L)	3.0%	25 min.	4.7	10¢
SS White Powder Free, 740-(XS-S-M-L)	2.8%	24 min.	4.6	11¢
MedPac Powder Free, 2000-(S-M-L)	5.1%	30 min.	4.9	10¢
Safeskin Satin Plus Powder Free, SP2-(XS-S-M-L-XL)	3.4%	25 min.	4.5	13¢
Safeskin Powder Free, 6301097-(XS-S-M-L)	2.7%	21 min.	4.6	12¢
Bio-Flex Gel Coat, BPF-(XS-S-M-L)	2.4%	19 min.	5.3	10¢
Life Guard Powder Free, 120-(S-M-L)	3.4%	20 min.	4.5	9¢
Sime Powder Free, 2000-(XS-S-M-L-XL)	0.7%	11 min.	4.5	6¢
Safetex P.F., 104-930-03-(S-M-L-XL)	2.1%	13 min.	4.8	11¢
Defend Latex Powder Free, 16-211-(XS-S-M-L)	5.1%	17 min.	4.5	11¢
Textured Powder Free, 18PFT-(S-M-L)	5.8%	17 min.	4.5	9¢
Dash Powder Free, (no order #)-(XS-S-M-L)	2.1%	2 min.	5.1	8¢
Arista Powder Free, PF208-(XS-S-M-L-XL)	4.2%	3 min.	4.5	8¢
Cranberry Powder Free, 16-201-(XS-S-M-L)	6.4%	9 min.	4.6	9¢
Exam Plus Powder Free, BS03-(XS-S-M-L-XL)	5.1%	2 min.	4.4	9¢
Skin-Gard P.F., 070-(XS,S-M-L)	10.8%	12 min.	4.9	7¢
Partners Powder Free, GWZS-(S-M-L)	10.8%	2 min.	4.5	6¢
PM Vinyl Powder Free, PMVPF-(S-M-L-XL)	— §	0 min. ¶	3.8	9¢
Durasafe Vinyl Powder Free, 223-(S-M-L-XL)	— §	0 min. ¶	3.3	9¢
Defend Vinyl Powder Free, 18-121-(S-M-L)	— §	0 min. ¶	3.9	12¢

§ FDA Water Leak Test does not discriminate defects in vinyl gloves.
¶ Vinyl is porous & allows immediate hydration
* On scale of 1-7, 7=BEST

BEST GLOVES OVERALL ARE Biogel-D, Crosstex Powderless, Ansell Conform, Golden Glove & Aladan Tri-Clean 110. LEAST EXPENSIVE, BEST OVERALL GLOVE is Golden Glove.

2. CLINICAL & LAB DATA THAT STRONGLY IMPLICATE GLOVE DEFECTS IN VIRUS TRANSFER.

PREMISE: Gloves are worn by clinicians to prevent exposure of patient & clinician to each other. *Integrity* of the barrier is first concern.

> *IN VIVO* LEAKS DEMONSTRATED: A Los Angeles surgeon who infected 18 of 142 patients (13%) in 10 months with hepatitis B theorized that finger irritation & pressure from suture tying might be implicated in virus transmission. Tests showed hepatitis B virus in washings from *inside* of gloves he wore while tying sutures, indicating he shed virus through skin on his fingertips. These viruses (42 nm), shed through irritated skin & escaping through defects in glove fingers provide first plausible explanation of disease transfer between patients & a clinician practicing Universal Precautions. (New York Times, March 22, 1994, C3)

> *IN VITRO* LEAKS DEMONSTRATED: Investigators testing 240 latex & 240 vinyl exam gloves found leakage of test virus ϕX174 in both types of gloves, regardless of whether gloves were just removed from box or stressed in 3 levels of simulated use. At heaviest use level, vinyl gloves leaked virus 9 times more frequently than latex gloves tested. (Korniewicz, D.M. et al., J Clin Micro 1990; 28:787-788)

****WITHOUT EFFICACIOUS INFECTION CONTROL PRODUCTS, THERE IS NO CONTROL OF INFECTIOUS DISEASES.**

CAUTIONS: A. Chlorination treatment is most common way to make powder free gloves. Process is *conceptually* good because it lowers protein antigens. In practice it is often done poorly to save time & money, & results in shortened shelf life, increased defects, slippery surface, foul taste & smell on gloves, brittle & cracking fingernails on clinician.

B. Vinyl is *porous*.

***CHOOSE POWDER FREE GLOVES CAREFULLY. STORE COOL, & USE WITHIN 3-4 MONTHS.*

3. CRA CONCLUSIONS:

Dental clinicians need to be aware that routine use of operating gloves can result in various hypersensitivity symptoms. Prevention is superior to searching for solutions after symptoms develop. New option is *powder free* gloves. Outstanding gloves in evaluation are listed above under BEST GLOVES.

REFERENCE NO. 7

VOLUME 16, ISSUE 10 – OCTOBER 1992
CRA Newsletter

SUBJECT: GLOVES, NON-STERILE OPERATING – UPDATE REVIEW

One year ago, CRA reported on quality & cost of 6,000 non-sterile operating gloves representing 20 brands used by dental clinicians (see Sep. '91 CRA Newsletter). Only 4 of 20 brands tested had *combination* of reasonable cost, overall good clinical characteristics, & low defects (7 of 20 brands met FDA's specification of 4.0% or less defects). Since both clinicians & patients depend on operating glove quality as primary infection control barrier, 1 year later the same tests were performed on all gloves still available to check for improvements. Following report is written in style similar to '91 report to facilitate comparisons.

1. TEST METHODS.

(a) GLOVE PURCHASE – Three boxes containing 100 gloves each of 24 brands of operating gloves were purchased from 36 distributors in 12 states in April, May, June '92. These included brands from the '91 evaluation, several new brands, & gloves from 1 manufacturer sold by 3 different distributors.

(b) CLINICAL CHARACTERISTICS TESTING – Seven characteristics evaluated were: (1) taste, (2) powder amount & texture, (3) tackiness after wetting, (4) tactile sensitivity, (5) resistance to tearing, (6) fit, & (7) cuff length & width.

(c) MANUFACTURING DEFECTS TESTING – FDA *in vitro* Water Tight Test was performed on 7,006 gloves to identify manufacturing defects. (All 7,200 gloves expected in this test were not available for testing due to presence of less than 100 gloves in 24 of the 72 boxes. One box contained only 54 of expected 100 gloves!)

2. **RESULTS.**

Refer to chart on page 2 for brand names that correspond to number abbreviations below & for details of evaluation.

(a) Gloves with **best *combination*** of reasonable cost, good clinical characteristics, & low manufacturing defects (pinholes) were defined as having cost of 8 cents or less, overall "good" clinical rating, & 4% or less defects. By these criteria #'s 1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 13, & 17 were noteworthy.

(b) Gloves with **best *combination*** of above characteristics both in '92 & '91 were #'s 1 & 3.

(c) Gloves with **least manufacturing defects** (pinholes) were #'s 1, 4, 9, & 12.

(d) Gloves with **least cost (6 cents/glove)** were #'s 1, 2, 3, 5, 11, 15, & 18.

(e) Comparing '92 to '91 evaluation, following was observed:

(1) Significantly fewer manufacturing defects were observed (i.e. 2.5% or 176 total defective gloves out of 7,006 tested in '92 vs. 6.6% or 392 total defective gloves out of 5,937 tested in '91).

(2) Clinicians desiring gloves with 4.0% or less manufacturing defects (FDA maximum allowable) have more brands from which to choose in '92 (i.e. 19 in '92 vs. 7 in '91).

(3) Clinicians desiring gloves with overall **best *combination*** of reasonable cost, good clinical characteristics, & least manufacturing defects have more brands from which to choose in '92 (i.e. 12 in '92 vs. 4 in '91).

(4) No gloves were rated excellent in all clinical characteristics in '92 (i.e. 0 in '92 vs. 2 in '91).

3. **OBSERVATIONS:**

(a) More boxes of gloves lacked the expected 100 gloves than noted in previous tests (24 of 72 boxes). Most problematic were glove #'s 1, 10, 12, & 15.

(b) Cuffs on about half of gloves tested were too short to remain over fitted uniform sleeve to provide continuous barrier from hand onto arm.

(c) Some glove designs have recognized need for anatomically placed thumb on non-sterile gloves to relieve pressure on hand (glove #'s 17 & 21). Glove 23 was nitrile rubber rather than latex, which conforms to hand better & relieves pressure.

(d) Many glove brands now make "hypoallergenic" claim. CRA questions whether any glove made from latex can be truly hypoallergenic. In future, FDA may set

standards for gloves making this claim, but currently no standards exist.

(e) Since manufacturing defects continue to be a consideration, methods are needed for clinicians to test gloves before use &/or apply something to gloved hands to seal surface.

4. CRA CONCLUSIONS:

Overall quality of non-sterile operating gloves tested has improved substantially over last year. Twelve of 24 brands had combination of reasonable cost, good clinical characteristics, & lowest manufacturing defects – a 30% improvement over '91 test results. One glove (S.S. White Latex Dental Exam Gloves) had *no* manufacturing defects in 300 gloves tested, which demonstrates holes in gloves are not inevitable. CRA urges all glove manufacturers to strive for consistent zero defects & improve clinical characteristics.

5. GLOVES TESTED‡

GLOVE NAME & ORDER NUMBER‡	SUGGESTED RETAIL COST	CLINICAL RATING§	GLOVE DEFECTS	OVERALL RATING‡
*1. Bio-Flex Dental Examination Gloves #BIO	$5.95/100 Case of 20 boxes (6¢/glove)	Good	1.0%	3.0
2. Ammex Latex Exam Gloves #AM721	$5.95/100 Case of 10 boxes (6¢/glove)	Good	1.7%	3.2
*3. APO Health Non-Sterile Latex Examination Gloves #Natural Color	$6.00/100 Case of 20 boxes (6¢/glove)	Good	2.0%	3.3
4. Ammatex DispoMed Exam Gloves #LX-102	$7.50/100 Case of 10 boxes (7.5¢/glove)	Good	1.0%	3.5
5. Sime Disposable Examination Gloves #10001 10003	$5.95/100 Case of 20 boxes (6¢/glove)	Good	2.4%	3.5
6. "Audra Gloves You" Disposable Latex Gloves #1	$7.50/100 Case of 10 boxes (7.5¢/glove)	Good	1.3%	3.6
7. RubberCare Latex Examination Gloves #8050	$6.90/100 Case of 10 boxes (6.9¢/glove)	Good	2.0%	3.6
8. Glovetex Latex Examination Gloves #1012	$6.45/100 Case of 10 boxes (6.5¢/glove)	Good	2.4%	3.6
9. S.S. White Latex Dental Exam Gloves #95001	$8.00/100 Case of 10 boxes (8¢/glove)	Fair	0.0%	3.7
10. Aladan Latex Medical Gloves #1002	$7.50/100 Case of 10 boxes (7.5¢/glove)	Good	1.7%	3.7
11. Silky Touch Hypoallergenic Latex Examination Gloves #LG-ST-Med	$5.95/100 Case of 10 boxes (6¢/glove)	Good	3.1%	3.7
12. Healthco Disposable Latex Examination Gloves #HCOG101001 & HCOG101011	$7.50/100 Case of 20 boxes (7.5¢/glove)	Fair	1.0%	3.8
13. Pharmaseal Flexam Floor/Exam Latex Gloves #8857	$7.28/100 Case of 20 boxes (7.3¢/glove)	Good	2.4%	3.9

14. Ansell Disposable Latex Examination Gloves #HCOG100011 & HCOG100021	$6.50/100 Case of 20 boxes (6.5¢/glove)	Fair	2.4%	4.0
15. Chatsworth Latex Examination Gloves #8000 & 8001	$5.95/100 Case of 20 boxes (6¢/glove)	Good	4.2%	4.1
16. Safeskin Hypoallergenic Latex Examination Gloves #300 & 200	$7.45/100 Case of 10 boxes (7.5¢/glove)	Fair	2.0%	4.2
17. Naturalfit Latex Dental Exam Gloves No order #	$9.45/100 Case of 20 boxes (9.5¢/glove) (Canadian cost)	Good	1.7%	4.4
18. Columbia World Latex Examination Gloves #C102	$5.99/100 Case of 10 boxes (6¢/glove)	Fair	4.4%	4.5
19. Defend Hypo-Allergenic Latex Examination Gloves #LG-1003 & LG-1004	$6.95/100 Case of 20 boxes (7¢/glove)	Good	4.8%	4.6
20. Micro-Touch Latex Medical Gloves #5741 & 5721	$10.67/100 Case of 10 boxes (10.7¢/glove)	Fair	1.3%	5.0
21. QualiTouch Left/Right Latex Exam Gloves #40-865	$11.95/100 Case of 10 boxes (12¢/glove)	Good	3.1%	5.7
22. Protector Latex Examination Gloves #EX-2	$6.50/100 Case of 10 boxes (6.5¢/glove)	Good	8.7%	5.7
23. Nitril N-Dex Gloves #6005M	$15.00/100 Case of 20 boxes (15¢/glove)	Good	2.4%	6.5
24. Natraflex Ambi Latex Exam Gloves #8881-498706	$15.50/100 Case of 10 boxes (15.5¢/glove)	Fair	4.8%	7.8

‡ Overall rating is result of averaging across cost, 7 clinical characteristics, & mean manufacturing defects in 3 boxes of gloves. Low number = best grade.

* Indicates gloves from both '91 & '92 evaluations that had combination of reasonable cost, overall good clinical characteristics, & low manufacturing defects.

§ Each of 7 clinical characteristics listed on page 1, item 1 (b) were graded separately (Excellent=1, Good=2, Fair=3, Poor=4) & an overall mean determined.

CAUTION: Be careful if attempting to purchase gloves on above chart if purchasing from distributors other than those listed because gloves may not be same as those tested by CRA.

REFERENCE NO. 8

VOLUME 15, ISSUE 9 – SEPTEMBER 1991
CRA Newsletter

SUBJECT: GLOVES, NON-STERILE LATEX

Use of operating gloves by dental clinicians has become standard practice in U.S. AIDS epidemic & recent government regulations (OSHA) are factors responsible for shift from bare hands to gloves. Today, question is not "Should I wear gloves?" but "*WHICH GLOVES* should I wear?". Choices are based heavily on cost, but clinical characteristics also influence decision. However, most important factor – *absence of holes* – generally is not considered because clinicians have no way to detect small defects called pinholes, where molten latex failed to fuse during manufacturing process. U.S. government (FDA) has set 4% as maximum pinhole defects allowable in non-sterile latex gloves. Unfortunately, FDA tests that detect pinholes render gloves unsuitable for clinical use. To identify glove brands with least pinholes, most desirable clinical characteristics, & most reasonable cost, CRA purchased 6,000 gloves from all over U.S. & subjected them to testing. Below report includes: (1) test methods; (2) results & observations; (3) CRA recommendations on glove use; (4) gloves tested (page 2 chart); & (5) CRA conclusions.

1. TEST METHODS.

To consider 3 areas of interest to clinicians concerning gloves (cost, clinical characteristics, defects [pinholes]), CRA:

(a) Surveyed 10,000 dental clinicians to determine most popular gloves & most common sources for purchase (Dec. '90 CRA Newsletter).

(b) Purchased 6,000 gloves from 18 distributors located in 13 U.S. states.

(c) Tested clinical characteristics (taste, powder amount & texture, tackiness after wetting, tactile sensitivity, resistance to tearing, & fit) in controlled in-house & field tests.

(d) Performed *in vitro* test for manufacturing defects prescribed by FDA (Water Tight Test) on 20 different glove brands using 100 gloves from each of 3 different boxes. Every attempt was made to secure 3 different lot numbers. Lot distribution among brands was: 11 with 3 different lots, 2 with 2 different lots, & 6 with only 1 lot. Gloves were tested in multiple replicates (1 replicate = 1 glove from each of 60 boxes in random order).

2. RESULTS & OBSERVATIONS.

Chart on page 2 gives details of evaluation. Below is summary of results:

(a) Gloves with ***combination*** of low pinhole defects, most of desirable clinical characteristics, & reasonable cost were #'s 2, 3, 5, 6 on page 2 chart.

(b) In pinhole defects, 8 of 20 glove brands tested met FDA specifications of 4% or less. They are listed as #'s 1-8 on page 2 chart.

(c) In clinical characteristics, 3 of 20 glove brands were rated excellent (#'s 1, 2, 5 on page 2 chart), 12 were rated good (#'s 3, 6, 8, 9, 10, 11, 12, 15, 17, 18, 19, 20 on page 2 chart), & 5 were rated fair (#'s 4, 7, 13, 14, 16 on page 2 chart).

(d) Left/Right Latex Exam Gloves sold by Smart Practice had least defects, best quality across lots, & most novel design. This was only glove in study constructed on "clutched hand" mold rather than flat hand mold. Purpose of this innovation is to relieve continuous stress on hand muscles when gloved fingers are in working position on instruments.

(e) Nitrile N-Dex Gloves sold by Safeware Supplies was only glove in study made of nitrile rubber rather than latex. Although it did not pass FDA specifications for maximum pinhole defects in new gloves with 8.3% defects, tests showed it was considerably more resistant to puncture during use.

3. CRA RECOMMENDATIONS ON GLOVE USE.

(a) Wash hands well before putting gloves on & between glove uses to lower microbe counts. Warm, moist environment created by gloves accelerates microorganism activity on skin. Use antiseptic demonstrated rapid antimicrobial activity plus residual & cumulative action. CRA tests show 4% chlorhexidine gluconate formulations meet these criteria best. Some brand names include Bactoshield, CHG, Excelle, Hibiclens, Luroscrub, Novoclens, & Steri-Stat.

(b) Wear long sleeved uniform & purchase gloves with long enough cuff to allow gloving *over uniform sleeve*. Purpose of gloves & uniform is to provide barrier between environment & bare skin. Bare arms compromise this goal.

(c) Today, patients want assurance operator's gloves are used only in their treatment. Plan to glove & deglove in front of each patient.

(d) Operating gloves should not be used for operatory cleanup because design that allows tactile sensitivity is not as resistant to puncture as utility gloves.

4. GLOVES TESTED

* Order of best pinhole percent. ** Pg. 1, section 1, item (c) lists characteristics considered.

GLOVE NAME & ORDER NUMBER*	COST PER GLOVE FROM CRA SOURCE	CLINICAL RATING **	PERCENT DEFECTS IN 300 GLOVES
1. Left/Right Latex Exam Gloves #40870 (Clutched Hand Mold)	$11.95/100 12¢ each Case of 10 boxes of 100	Excellent	0.7
2. Non-Sterile Latex Examination Gloves #APOMW	$6.00/100 6¢ each Case of 20 boxes of 100	Excellent	1.3
3. Latex Exam Gloves now called Bio-Flex #B1000	$6.00/100 6¢ each Case of 20 boxes of 100	Good	2.0
4. Natraflex Ambl Latex Exam Gloves #8881-498706	$12.30/100 12¢ each Case of 10 boxes of 100	Fair	2.4
5. Micro-Touch Latex Medical Gloves or Dental Gloves #5721 or 5720-00	$8.50/100 9¢ each Case of 10 boxes of 100	Excellent	2.7
6. All Latex Multi-Purpose Ambidextrous Disposable Gloves #C-102	$5.99/100 6¢ each Case of 10 boxes of 100	Good	2.7
7. Defend Latex Examination Gloves #LG-1004	$8.95/100 9¢ each Case of 20 boxes of 100	Fair	3.7
8. Disposable Latex Examination Gloves #HCOG100021 & HCOG100011	$7.95/100 8¢ each Case of 20 boxes of 100	Good	4.3
9. Pharmaseal Flexam Floor/Exam Gloves #8857	$5.99/100 6¢ each Case of 10 boxes of 100	Good	5.4
10. Glovetex Disposable Ambidextrous Natural Latex Examination Gloves #3030	$6.45/100 7¢ each Case of 10 boxes of 100	Good	5.7
11. Disposable Latex Examination Gloves— Ambidextrous Unisize #HCOG101001	$7.95/100 8¢ each Case of 20 boxes of 100	Good	6.0
12. Latex Medical Gloves #1002	$7.95/100 8¢ each Case of 10 boxes of 100	Good	6.7
13. Disposable Examination Gloves #SL02	$5.95/100 6¢ each Case of 20 boxes of 100	Fair	7.0
14. Latex Examination Gloves No order #	$5.95/100 6¢ each Case of 10 boxes of 100	Fair	7.7
15. Nitrile N-Dex Gloves #7005	$9.80/100 10¢ each Case of 20 boxes of 100	Good	8.3
16. Latex Examination Gloves #M-1002	$5.00/100 5¢ each Case of 20 boxes of 100	Fair	9.3
17. Latex Examination Gloves #R1001	$8.95/100 9¢ each Case of 20 boxes of 100	Good	10.3
18. Disposable Non-Sterile Latex Examination Gloves #XGM	$5.90/100 6¢ each Case of 10 boxes of 100	Good	13.1
19. Disposable Latex Gloves No Order #	$7.50/100 8¢ each Case of 10 boxes of 100	Good	15.2
20. Latex Examination Gloves #8001	$5.90/100 6¢ each Case of 20 boxes of 100	Good	17.4

*CAUTION: Be careful if attempting to purchase gloves on above chart if purchasing from distributors other than those listed because gloves may not be same as those tested by CRA. Glove industry has complicated interwoven network that makes glove manufacturing source & grade impossible for clinicians to trace. To link responsibility for glove quality to manufacturing source, name & address of manufacturer should be listed on each box, but currently this is not required.

5. CRA CONCLUSIONS:

Gloves with *combination* of consistently low pinhole defects, most of desirable clinical characteristics, & reasonable cost are listed in chart above under following numbers: 2, 3, 5, 6. Most gloves tested (12 of 20 brands) exceeded 4% defects specified by FDA, indicating need for clinical method to test for defects *before* using gloves or material to spread over gloved hands to seal latex surface before use. Currently double gloving is only way to manage glove defect problem.

REFERENCE NO. 9

VOLUME 13, ISSUE 1 – JANUARY 1989
CRA Newsletter

SUBJECT: OPERATING GLOVES, UPDATE

STATUS OF DENTAL GLOVES IN U.S. Operating gloves are now used routinely by many dental clinicians. Primary factor in change from bare to gloved hands was AIDS epidemic which stimulated increased awareness of infectious diseases in general. Unexpected demand for gloves created shortages, increased prices, & decreased quality. However, currently supply is surpassing demand, & cost & quality are improving. In future, government regulations will stipulate criteria for both sterile & non-sterile glove quality.

GLOVES SELECTED FOR CRA EVALUATION. Glove origin must be identified by consumer to determine glove differences. This is difficult because a few manufacturers supply a large number of distributors. In order to conceal fact that same glove is sold under many different brand names for many different prices, name of glove manufacturer is generally kept confidential & each distributor uses unique brand name &/or label designs. This practice has serious implications for clinicians having hypersensitivity & fit problems. It also prevents check to ascertain if glove cost is commensurate with quality. To overcome these communication obstacles, chart on page 2 lists gloves tested alphabetically by *manufacturer's name.*

GOALS OF EVALUATION. CRA goal for this evaluation was to test gloves sold by well known distributors that represented all 6 major U.S. manufactures (Aladan, Ansell, Baxter/Travenol, Perry, Surgikos, Tillotsen), plus

manufacturers outside U.S. To locate your particular gloves in chart on page 2, call distributor where you purchased the gloves & obtain *manufacturer's name.* Refusal to disclose name of manufacturer generally indicates problems with quality or pricing.

CHARACTERISTICS TESTED & PROCEDURES USED. Glove characteristics tested were: (1) Manufacturing defects using 2 different methods (TEST METHOD 1 – ASTM Airtight Test, ANSI/ASTM D 3577-78a. Glove inflated with air [1.5kPa or ~ 0.657 psi] & submerged in H_2O so longest finger is about 200 mm from surface & tester checks for escaping air. TEST METHOD 2 – Ballbach Air Inflation Test, Ballbach R.L., et. al., J AOAC, 55(5)1074-1080, 1972. Uses protocol similar to ASTM Airtight Test except glove is compressed to force air into one finger at a time which places glove material under tension while tester checks for escaping air); (2) Smell; (3) Taste; (4) Amount of powder; (5) Tactile sense; & (6) Resistance to tear. Test results are reported in chart on page 2.

CRA OVERALL CONCLUSIONS:

Gloves with comparatively good quality were identified in all 5 categories tested. This information provides clinicians with tenable basis for glove selection. Clinical characteristics such as smell, taste, amount of powder, tactile sense, & tear resistance need improvement in most gloves tested. Chart on page 2 links names of manufacturers & distributors to specific gloves. This assists clinicians with hypersensitivity & fit problems to identify gloves with actual differences. Two brands of vinyl gloves tested demonstrated quality that enables individuals hypersensitive to latex gloves to consider

them as viable alternative. One copolymer over-glove had very low number of defects, making it possible to consider over-glove as way to avoid contamination when touching telephone, patient chart, x-rays, pencils, or pens, etc., during patient treatment.

EVALUATION OF OPERATING GLOVES

A	B	C	D		E				
MANUFACTURER	COUNTRY CONTROLLING PRODUCTION	COST PER GLOVE FROM REPRESENTATIVE U.S. DISTRIBUTOR	ASTM PERCENT DEFECTS *	BALLBACH PERCENT DEFECTS *	SMELL * *	TASTE * *	AMOUNT OF POWDER * *	TACTILE SENSE * *	RESISTANCE TO TEAR * *
LATEX, NON-STERILE									
1. Aladan	U.S.	16¢	0.7	15.1	F	G	E	G	P
2. Ansell	U.S.	13¢	1.6	9.4	G	F	G	G	F
3. Bantex Corp.	Taiwan	13¢	16.0	38.0	G	G	G	F	G
4. Baxter/Travenol	U.S.	15¢	2.3	8.0	G	G	G	E	E
5. Baxter/Travenol	U.S.	18¢	2.1	9.6	G	G	G	G	E
6. London Rubber Co	England	48¢	2.3	7.3	G	P	E	F	G
7. Perry	U.S.	20¢	4.0	9.0	G	P	G	P	G
8. Surgikos	U.S.	30¢	2.0	6.5	F	G	F	G	G
9. Surgikos	U.S.	19¢	3.0	8.3	G	F	F	G	G
10. Tillotsen	U.S.	13¢	1.0	16.7	F	F	G	G	P
11. Unidentified	Taiwan	—	41.3	65.2	E	G	F	G	P
12. Unidentified	China	—	8.0	28.0	G	G	G	G	P
13. Unidentified	Malaysia	13¢	3.3	15.3	G	G	G	G	P
LATEX, STERILE									
1. Baxter/Travenol	U.S.	35-40¢	1.5	5.5	G	G	G	G	G
HYPOALLERGENIC, STERILE									
1. Baxter/Travenol	U.S.	63¢	5.1	17.5	P	G	G	G	F
2. Baxter/Travenol	U.S.	$1.10	2.7	9.2	G	G	G	G	G
VINYL, NON-STERILE									
1. Baxter/Travenol	U.S.	10¢	NT	1.0†	G	G	E	E	G
2. Becton-Dickinson	U.S.	10¢	NT	2.0†	F	G	E	F	E
3. Becton-Dickinson	U.S.	11¢	NT	6.0†	F	F	E	G	G
COPOLYMER, NON-STERILE									
1. Armin Poly-Version	U.S.	5¢	NT	4.0†	E	E	E	E	—
2. Becton-Dickinson	U.S.	7¢	NT	9.0†	E	E	E	E	—
3. Unidentified	China	5¢	NT	11.1†	E	E	E	E	—
4. Unidentified	Taiwan	4¢	NT	25.3†	E	E	E	E	—

* Lots tested available upon request.

NT Not tested using ASTM Airtight Test because lack of elasticity precluded mounting procedures used in this method.

* * E= excellent; G= good; F=fair; P= poor

† Agreement has not been reached on standard test for these gloves. Defect percentages using Ballbach Air Inflation Test are deceptively low because glove material does not have same elasticity as latex & air compressed into fingers is under lower pressure.

TEST RESULTS BY GLOVE TYPE:

1. LATEX GLOVES – Most gloves tested met, or came close to meeting, ≤2.5% manufacturing defects using ASTM Airtight Test. However, if latex was stressed (Ballbach Air Inflation Test), percent manufacturing defects on all gloves tested increased *significantly*, indicating presence of weak spots &/or smaller holes not detected by ASTM procedure. Generally, best gloves had low ASTM & Ballbach percentages, & Ballbach percentages were only 3-4 times higher than ASTM percentages (ie: gloves listed alphabetically on chart above as Ansell, Baxter, London Rubber, & Surgikos). Some imported gloves came close to this criteria (Malaysia), but gloves tested from Taiwan & China had substantially higher number of defects. Clinical characteristics of all gloves tested need improvement.

2. VINYL GLOVES – Brands of vinyl gloves tested had significantly fewer defects than those tested by CRA in past using same method. However, test method used could be questioned because standard test for vinyl gloves is lacking. Baxter/Travenol & Becton-Dickinson Tru-Touch gloves could be alternatives for clinicians hypersensitive to latex gloves.

3. COPOLYMER OVER-GLOVES – Quality of Armin Poly-Version copolymer gloves was significantly better than expected for this type manufacturing process. These gloves could be useful worn over operating gloves during non-treatment tasks required during patient treatment such as handling x-rays, patient chart, telephone, etc. Gloved hands should be washed well before touching over-gloves to minimize cross-contamination.

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Leakage of Virus through Used Vinyl and Latex Examination Gloves

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A total of 480 examination gloves (240 vinyl and 240 latex) were stressed by using manipulations designed to mimic patient care. At the highest use level, 38 (63%) of 60 vinyl gloves leaked bacteriophage ϕX174 compared with 4 (7%) of 60 latex gloves. At lower use levels, there was no statistically significant difference in leakage.

Recently, there has been concern among health care personnel about the use of vinyl and latex gloves as barriers against the transmission of microorganisms. A recent outbreak of herpes whitlow among intensive care nurses who wore gloves[7] has focused attention on the integrity of gloves during use. We previously investigated vinyl and latex examination gloves as barriers against bacteria and found that both types of gloves provided some protection, but latex gloves maintained

integrity longer under in-use conditions.[6] Dalgleish and Malkovsky[2] have reported that the quality of latex gloves varies with the manufacturer and that latex gloves may not give total protection from human immunodeficiency virus penetration. In fact, gloves scanned by electron microscopy have shown 30- to 50- μm holes, suggesting that viruses could penetrate the gloves.[1] Degroot-Kosolcharoen and Jones[3] investigated the permeability to water and blood of sterile latex gloves and examination latex and vinyl gloves and found greater leakage in examination gloves. Health care personnel continue to question the barrier effectiveness of latex and vinyl examination gloves used in clinical practice. This study was conducted to determine whether bacteriophage ϕX174 could penetrate used vinyl and latex examination gloves after standardized manipulations.

Procedure for glove manipulation. Because of findings in our previous study (6), standardized manipulations designed to mimic patient care activities were performed on groups of gloves in advance of testing (Table 1). Participants wore no rings or nail polish and had their fingernails filed short and smooth. Sixty gloves of each type were tested at each use level. As positive controls, vinyl and latex gloves had either two or five holes punctured in the index finger with a 21-gauge hypodermic needle and were assayed for bacteriophage leakage. In addition, one latex glove was torn approximately 1 cm at the index finger.

Preparation and assay of phage. Stock suspensions of ϕX174 bacteriophage were prepared by growth in liquid culture with *Escherichia coli* C (ATCC 13706) at 37°C (5). The titer of ϕX174 was determined by plaque formation

using the tryptone top-agar layer method (5, 8). Phosphate-buffered saline (100 ml; pH 7.4) containing10^6 to 107 PFU of ϕX174 was poured into each glove. The quantity of liquid was enough to fill the glove to just about the fingers. Each glove was held over an empty collection container for 1 min and observed for leakage of liquid. Each glove was then draped for 4 min over the edge of a collection container such that the fingers were suspended in the interior of the container and the empty remainder of the glove fell over the exterior. Phosphate-buffered saline (100 ml) was poured into the collection container, and the fingers were immersed for 5 min more. Samples (1ml) of the collection buffer were obtained after up-and-down agitation and removal of the glove. In this study, a visible leak was defined as a leak observed by the naked eye. A viral leak was one in which virus was detected in the collection container. The effective volume leaked from each glove was calculated in microliters as the total number of virus particles in the collection container divided by the virus concentration inside the glove. The chi-square and the Fisher exact tests were used to assess differences in the proportions of gloves which leaked. The Mann-Whitney U test was used to test for differences in the volume of fluid leaked.

A total of 480 (240 vinyl and 240 latex) examination gloves were tested, with 60 each in the four categories of use. None of the latex gloves with needlestick holes had visible leaks or were positive for viral leakage. However, the torn latex glove was positive for both visible and viral leaks (5.0×10^6 PFU). The punctured vinyl gloves had both visible and viral leakage (two holes, 7.6×10^3 PFU; five holes, 4.9×10^6 PFU). All test gloves with visible

leaks also leaked virus (Fig.1). Virus leakage was found in both types of gloves: 55 (22.9%) of 240 vinyl and 18 (7.5%) of 240 latex gloves ($x^2 = 20.94$. $P = 0.00005$). In addition, in 34 (14.1%) of 240 vinyl gloves and 16 (7%) of 240 latex gloves, visible leaks were not observed, but virus still leaked through: 5×10^2 to 1×10^6 PFU per glove for vinyl and 5×10^2 to 1.3×10^4 PFU for latex.

There were not statistically significant differences in leakage rates for latex or vinyl gloves at use level 0, 1, or 2. However, as with visible leaks, there was a statistically significant difference in viral leaks at use level 3. Of the vinyl gloves, 63% (38 of 60) in use level 3 leaked virus compared with 7% (4 of 60) of the latex gloves (Fisher exact test, two tailed; $P = 0.000001$). There was a wide range in the volume leaked at each of the four use levels (Table 2). Vinyl gloves leaked more than latex gloves (Mann-Whitney U test: $P = 0.0001$).

This study demonstrated that some vinyl and some latex examination gloves permit viral leakage. At use levels 0, 1, and 2, the proportions with viral leakage were not significantly different between glove types. At use level 3, more vinyl gloves than latex gloves leaked virus, and the volume leaked was higher. The needlestick punctures in the fingertips of gloves which were meant to serve as positive controls resulted in visual and viral leaks in the vinyl gloves but not in the latex gloves. This result was unexpected and may have been caused by the elastic resealing property of latex.

The high proportion of gloves that leaked some amount of ϕX174 indicates that the effective hole size is greater than 27 nm, since that is the diameter of the virus

particle. These findings are consistent with the work of Kotilainen et al. who were able to detect leakage of poliovirus (20 to 30 nm in diameter) through a glove barrier. Further evidence suggests that microorganisms can pass from the outside of a glove, through the glove, and onto the hand.[4,6]

These results, from the testing of a single brand of vinyl gloves, suggest that holes can be formed during procedures related to routine patient care. Under the conditions of this study, latex gloves appeared to be less susceptible to hole formulation than vinyl gloves. Although these experiments should be expanded to include other brands of both types of gloves, our results suggest the need to change gloves after moderate periods of use. In addition, since there may be viral leakage during glove use, a conscientious handwashing technique should be used after glove removal.

Table 1. Procedures for preparation of gloves prior to test

Use Level[a]	Procedure
0	Remove from box
1	Don gloves and remove them
2	Levels 0 and 1 plus rub each gloved hand with a washcloth in the following sequence: palm each finger in a twisting motion, thumb, and back of hand
3	Levels 0-2 plus (I) attach a capped needle to a Luer-Lok syringe and then remove it 30 times; (ii) connect and disconnect

Luer-Lok syringe to intravenous tubing and manipulate a stopcock eight times; (iii) wrap, tape, and unwrap a blunt object two times to simulate bandaging an amputation stump

[a]Use level in previous paper refers to manipulation level (6).

Table 2. Volume leaked from vinyl and latex gloves

	Median leakage (μl: range)	
Use level	**Vinyl**	**Latex**
0	4.19 (0.34-8.03)	0.08 (0.02-227)
1	1.09 (0.08-1,250)	0.26 (0.02-0.54)
2	6.12 (0.11-1,250)	0.57 (0.03-840)
3[a]	229 (0.02-7,142)	2.20 (0.06-437)

[a]Mann-Whitney U-test differences in volume leaked between vinyl and latex gloves, $P = 0.000001$

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Survival of Hepatitis A Virus on Human Hands and Its Transfer on Contact with Animate and Inanimate Surfaces

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The survival of hepatitis A virus (HAV; strain HM175) on the hands of five volunteers was determined by depositing 10μl of fecally suspended virus on each fingerpad and eluting the inoculum after 0, 20, 60, 120, 180, and 240 min. The amount of virus recovered from each fingerpad at 0 min was approximately 6.0 x 10^4 PFU. At the end of 4 h, 16 to 30% of the initially recoverable virus remained detectable on the fingerpads. HAV inocula (10 μl; approximately 1.0 x 10^4 PFU) placed on fingerpads or 1-cm-diameter metal disks were used to determine virus transfer to clean surfaces upon a 10-s contact at a pressure of nearly 0.2 kg/cm^2. When the inoculum was dried for 20 min, virus transfer from fingerpad to fingerpad, fingerpad to disk, and disk to fingerpad ranged from 2,667 to 3,484 PFU, while 0 to 50 PFU could be transferred after 4 h of drying. Elevation of the contact pressure alone from 0.2 to 1.0 kg/cm^2

resulted in an approximately threefold increase in the amount of virus transferred. Incorporation of friction (10 half turns of the finger during 10 s of contact) with the low and high levels of pressure gave two- and threefold increased in the PFU of virus transferred, respectively. Pressure and friction were found to significantly affect HAV transfer ($F = 33.98$; $P < 0.05$), irrespective of the mode of transfer used. No statistically significant interaction was observed between mode of transfer and pressure or friction. The findings of this quantitative study suggest that human hands may play an important role in the direct as well as the indirect spread of HAV.

Infections caused by hepatitis A virus (HAV) are endemic throughout the world (6, 21, 41). Apart from community-based epidemics (7, 15, 16, 26), outbreaks of the disease are frequently associated with hospitals (3, 13, 25, 45), day-care centers (12, 15-17, 40), schools and institutions for mentally ill people (27, 31, 37, 41), and eating establishments (19, 25, 28, 30). Whereas fecally contaminated food (e.g., shellfish) and potable water are well recognized as vehicles for HAV (6, 8, 36, 41), in nearly 50% of the cases the vehicle(s) responsible for virus spread remains unidentified (15). Strong circumstantial evidence indicates that virus-contaminated hands play a major role in the spread of the virus, particularly in institutional settings (7, 19, 25, 26, 28, 30, 35).

For hands to be important HAV vehicles, the virus must remain viable on human skin. Previously, we have shown that HAV can survive on hard surfaces for prolonged

periods (33) and also that it is relatively resistant to many of the commonly available hard-surface disinfectants (32). Infectious virus transfer from contaminated to clean surfaces through contact has been reported (2, 14, 18, 20). Therefore, this study was designed to determine HAV survival on human hands and to assess how routine interactions of hands with other contaminated surfaces could further promote the spread of HAV. The study design also included determination of the role that inoculum age, pressure, and friction may play in the transfer of infectious HAV from contaminated to clean surfaces.

MATERIALS AND METHODS

Cells. A seed culture of FRhK-4 cells was kindly provided to us by M.D. Sobsey, University of North Carolina, Chapel Hill. The methods for the cultivation and maintenance of these cells have been described previously (32, 33, 47). Briefly, Eagle minimum essential medium (GIBCO, Grand Island, N.Y.) with 10% fetal bovine serum (GIBCO), 2 mM glutamine (GIBCO), 0.1 mM nonessential amino acids (GIBCO), 50μg of gentamicin sulfate (Cidomycin; Roussel, Montral, Quebec, Canada) per ml, 100 μg of kanamycin (GIBCO) per ml, 0.015 M HEPES (*N*2-hydroxyethylpiperazine-N′2-ethanesulfonic acid; GIBCO), and 0.113% sodium bicarbonate (BDH Chemicals, Toronto, Ontario, Canada) was used for growing the cells. The cells were maintained in the same medium, but the maintenance medium contained only 2% fetal bovine serum.

Virus. The HM-175 strain of HAV was also received from M.D. Sobsey. Stock virus was prepared by infecting

FRhK-4 monolayers at a multiplicity of infection of 0.01. The virus was allowed to absorb for 90 min at 37°C before the addition of maintenance medium and a further incubation at 37°C until 75 to 80% of each virus-infected monolayer was affected by virus cytopathology (4 to 5 days of incubation). The cultures were frozen (-20°C) and thawed three times, and the culture fluid was centrifuged for 10 min at 1,000 x g. The virus was concentrated 10-fold by polyethylene glycol hydroextraction as described by Ramia and Sattar (42). Briefly, dialysis tubing (Spectrum Medical Industries Inc., Los Angeles, Calif.) containing 100 ml of the clarified cell culture fluid was placed in a plastic tray and completely covered with polyethylene glycol (molecular weight, 8,000; Matheson, Coleman and Bell, Norwood, Ohio). After overnight hydroextraction at 4°C, the residue in the dialysis tube was resuspended in 2 ml of Earle balanced salt solution. The concentrate was divided into aliquots for storage at -70°C.

HAV plaque assay. Determinations of HAV PFU were carried out in FRhK-4 monolayers in 12-well plastic plates (Costar, Cambridge, Mass.) as described previously (32,33). Briefly, three wells were used for each virus dilution tested, and each well received 0.1 ml of the inoculum. HAV was then allowed to absorb for 90 min at 37°C. The overlay consisted of Eagle minimum essential medium, 2% fetal bovine serum, 2mM glutamine, 0.1 mM nonessential amino acids, 50µg of gentamicin sulfate per ml, 100 µg of kanamycin sulfate per ml, 2 µg of amphotericin B (Fungizone; GIBCO) per ml, 0.015 MHEPES, 0.113% sodium bicarbonate, 0.75% agarose (type II; Sigma Chemical Co., St. Louis, Mo.), and 26 mM

magnesium chloride (BDH). The plates were sealed in laminated plastic bags (Philips, Toronto, Ontario, Canada) and incubated for 8 days at 37°C. The procedure for fixing and staining the monolayers prior to counting plaques has been described previously (29).

Virus-suspending medium. The fecal samples used in this study was obtained from a healthy 5-month-old baby. A 10% (wt/vol) suspension of it was prepared in normal saline; it was clarified of gross particulate matter by centrifugation for 10 min at 1,000 x g and passed through a 0.2-μm-pore-size membrane filter (Nalge Co., Rochester, NY) to remove bacteria and fungi. The filtrate was found to be free of cytotoxicity and endogenous viruses when it was tested in FRhK-4 cells by the procedure described before (29). When HAV was diluted 1:10 in this fecal suspension and held at room temperature for 4 h, no loss in virus infectivity was observed.

Disks. Stainless-steel disks (diameter, 1 cm) were punched out of locally purchased no. 4 finish polished sheets (thickness, 0.75 mm) and were used to represent environmental hard surfaces. The procedure for their decontamination, cleaning, and sterilization before reuse has been described in detail before (44).

Volunteers. Permission to place the virus on the hands of adult volunteers was first obtained from our university's Ethics Committee. Any individual with cuts or abrasions on the hands was automatically excluded from the study. Each volunteer was then thoroughly briefed on the experimental protocol and the risks involved before being asked to sign a consent form. The age of the participants ranged from 26 to 45 years.

At the end of the experiment, the volunteer was asked to gently press the experimentally contaminated fingerpads on a piece of paper towel soaked in a 6% solution of sodium hypochlorite (Javex Canada Ind., Toronto, Ontario, Canada) for 3 min for their decontamination. The hands were then washed thoroughly with ordinary soap and running tap water and dried with a paper towel.

HAV survival on human hands. Virus survival on the hands of volunteers was tested by the procedure of Ansari et al. (2). The hands were first washed in lukewarm running tap water for 30 s, disinfected by thoroughly rubbing them with 70% ethanol (BDH), and then air dried. To demarcate the areas for virus deposition, each fingerpad was pressed hard over the mouth (8-mm inside diameter) of a empty plastic vial (no. 72.694.006; Sarstedt Inc., St. Laurent, Quebec, Canada).

Before each experiment, the stock virus was diluted 10-fold in the fecal suspension. Ten microliters of the fecally suspended virus was immediately placed, using a positive-displacement pipette (Gilson Medical Instruments, Villiers-le-Bel, France), into each of two glass vials containing 990 μl of Earle balanced salt solution; these acted as the input virus controls.

Ten microliters of the virus suspension was also deposited at the center of the demarcated area on each fingerpad. In each experiment, the amount of virus in the inoculum on the fingerpads (0-min control) was determined by immediately recovering the deposited virus separately from four fingerpads of the left hand, as follows. The contaminated area was placed over the mouth

of a vial identical to that used for fingerpad demarcation but containing 990 µl of Earl balanced salt solution. The vial was inverted, with fingerpad still over it, and held in position for 5 s. This was followed by 20 full inversions of the vial with the vial still in place and then an additional 5 s of soaking and 20 more full inversions. The surface of the fingerpad was then scraped on the inside rim of the vial to recover as much of the fluid as possible. This technique was found to recover approximately 6.0×10^4 PFU or 70% of the input infectious virus. To determine HAV survival on hands, virus was randomly eluted from separate fingerpads at 20, 60, 120, 180, and 240 min after their inoculation.

Five volunteer participated in the experiments on virus survival on hands, and each experiment was performed three times on each volunteer.

Contamination of disks and virus elution. Each clean and sterile disk received 10µl of fecally suspended HAV. In every experiment, the amount of infectious virus deposited on the disks (0-min control) was determined by placing two inoculated disks immediately into two separate glass vials containing 990 µl of Earle balanced salt solution. The vials were sonicated to elute the virus from the disks. This procedure recovered approximately 6.23×10^4 PFU, which represented nearly all of the infectious virus placed on each disk (33).

Pressure and friction. The effect of a low (approximately 0.2 kg/cm^2) and a high (approximately 1.0 kg/cm^2) level of pressure was tested in the virus transfer experiments. The low level of pressure, with or without friction, was taken to represent ordinary touching of

environmental surfaces. Animate-animate interaction through handshaking was represented by a pressure of 1.0kg/cm^2 without friction, while the same pressure with friction simulates opening of doors and other, equivalent actions.

Depending on the type of experiment, a clean or HAV-contaminated disk was placed at the center of the pan of a scale with a digital readout (model no. 1206 MP; Sartorius, Gottingen, Germany), and a virus donor or recipient fingerpad was pressed on it until the desired pressure was achieved and was held there for 10 s; pressure was computed from the balance reading and the area of the contaminated surface. For virus transfer from fingerpad to fingerpad, the recipient fingerpad was placed on the scale and placed into contact with the donor.

In an attempt to standardize the friction to be applied during virus transfer, each volunteer was first thoroughly briefed on and given the opportunity to properly practice the following procedure. Once contact was made between the disk and the fingerpad and the desired pressure level was attained, the finger was rotated in half circles 10 times over the 10-s period of contact; in the case of fingerpad-to-fingerpad transfer, the donor was required to apply friction.

HAV transfer experiments. The extent of infectious HAV transfer upon fingerpad-fingerpad interaction and fingerpad-environmental surface contact was examined as follows. After letting the virus inoculum dry on the donor surface of the desired length of time, contact was made at the required pressure, with or without friction, with the recipient surface for 10 s. Virus was then eluted

form the recipient as well as the donor surfaces. The two values thus obtained were added together to represent the total amount of infectious virus detectable on the donor before transfer, and the amount of virus transferred was determined as a percentage of this total. The disk-to-fingerpad and the fingerpad-to-disk experiments were conducted on each of two volunteers, whereas the fingerpad-to-fingerpad mode involved volunteer E as the donor and volunteer A as the recipient.

Statistical analyses. HAV survival on the fingerpad of each volunteer at each time point (x value) was expressed as a fraction of HAV eluted at 0 min. These observations were then normalized to the 0-min value as 100%. Such data were used to generate log-linear plots ($\log_{10}$) in a computer program (SigmaPlot version 4.0; Jandel Corp., Corte Madeira, Calif.). First-order linear regression lines were then fitted to the plots in the same program. HAV decay rates on the fingerpad were obtained from the gradient of the fitted regression lines as loss of virus infectivity titer in $\log_{10}$ PFU per minute. The half-lives were calculated from the decay rate constants (K_i), as discussed by Segel (46).

To analyze the data obtained from HAV survival and transfer between the three models (fingerpad-fingerpad, fingerpad-disk, disk-fingerpad) over 4 h by using two volunteers, the data were normalized as described above. Bar chart plots were then produced in the SigmaPlot computer program.

In order to assess the effects of pressure, friction, and the transfer mode used on the amount of HAV transferred,

the raw data were entered into an SAS program (Statistical Analysis Systems, University of North Carolina, Chapel Hill) and a three-way analysis of variance was performed. The possible interactions between the variables (pressure, friction, and type of model) were also analyzed. The Tukey test was performed post hoc in the same program. To compensate for differences in virus transfer because of time, an average of the values obtained with the 20- and 60-min HAV transfers was computed and used in the three-way analysis of variance.

RESULTS

HAV survival on human hands. The mean virus titer at 0 min on fingerpads in the HAV survival experiments was approximately 6.0×10^4 (range, 5.3×10^4 to 7.2×10^4) PFU in the 10-μl inoculum. The results of these experiments are presented in Fig. 1. The pattern of HAV decay on fingerpads appeared to be biphasic. Nearly 68% of the input infectious virus became undetectable within the first 60 min after contamination of the fingerpads. Even though this early loss in virus infectivity was particularly pronounced in volunteer E, the rate of decay was essentially similar during the 60- to 240-min period for all five volunteers. The amount of HAV detectable at the end of 4 h ranged from 16 to 30% of the initially detectable amount; the actual PFU recovered at the end of 4 h were 15,740 and 8,350 for volunteers D and E, respectively.

In view of the apparent biphasic nature of the loss in HAV infectivity on fingerpads, infectious virus half-lives and K_i values (expressed as the rate of loss in virus

infectivity in $\log_{10}$ per minute) were determined separately for the 0- to 240-min and the 60- to 240-min periods. The half-lives of the virus for both the periods were the shortest in volunteer E (5.50 and 14.44 h, respectively) and the longest in volunteer D (7.70 and 23.10H, respectively) (Table 1). However, the differences in virus half-lives and K_i values for the five volunteers were not found to be statistically significant either for the 0- to 240-min period or the 60- to 240-min period.

HAV transfer after 20 and 60 min of drying. The mean amounts of virus on the fingerpads and disks at 0 min were 3.1×10^4 and 2.9×10^4 PFU/ml, respectively. There was always virus transfer. A three-way analysis of variance revealed the significant main effects because of the mode of transfer, pressure, and friction ($F = 3.35$, $P < 0.05$; $F = 133.51$, $P < 0.05$; and $F = 81.29$, $P < 0.05$, respectively). A post hoc analysis (Tukey tests) of the main effect of the transfer mode showed a significant difference in virus transfer between the fingerpad-fingerpad (mean, 634.08 PFU) and the disk-fingerpad (mean, 454.72) modes. Similarly, there was a significant difference between the fingerpad-disk (mean, 607.21) and disk-fingerpad modes in the amount of virus transferred. The amount of virus transferred was not significantly different between the fingerpad-fingerpad and the fingerpad-disk modes.

The analysis also revealed significant differences in virus transfer because of pressure and friction. The increase in pressure from 0.2 kg/cm^2 resulted in a significant increase in the amount of virus transferred ($F = 133.51$, $P < 0.05$); the mean transfer rate went from 212.82 PFU at 0.2 kg/cm^2 to 917.85 PFU at 1 kg/cm^2. Similarly, the

incorporation of friction resulted in a significant elevation in the amount of virus transferred ($F = 81.29$, $P < 0.05$). The mean HAV transfer rate with no friction was 290.27 PFU, whereas with friction, the mean value was 840.40 PFU. The combination (interaction) of pressure and friction was also found to significantly affect the amount of HAV transferred ($F = 33.98$, $P < 0.05$). The two-way effect of mixing pressure and friction is illustrated in Fig. 2. The combination of friction and the higher pressure (1 kg/cm^2) resulted in a profound increase in HAV transfer (mean, 1,370.76). No statistically significant interactions were observed between mode of transfer and pressure and friction, mode of transfer and friction, or mode of transfer and pressure.

HAV transfer over a period of 4 h. The ability of HAV to be transferred over a 4-h period was assessed by using two volunteers (volunteers A and E). The three modes of virus transfer were tested by using only the low level of pressure without any friction. The mean amounts of virus at 0 min in the 10-μl inoculum on fingerpads and disks in these experiments were 1.1×10^4 PFU (1×10^4 to 1.2×10^4 PFU) and 1.08×10^4 PFU (1.07×10^4 to 1.09×10^4 PFU), respectively.

(i) Virus transfer from disks to fingerpads. Nearly 33% of the input virus PFU remained detectable on the disks at the end of 4 h under ambient conditions of temperature (22 ± 2°C) and relative humidity (45 ± 5%) (Fig. 3). When a clean fingerpad was pressed against a disk with an inoculum that was dried for 20 min, about 22% of the PFU (2,667 PFU) could be transferred, whereas no detectable virus was transferred to fingerpads when the inoculum was left to dry on the disks for 4 h.

(ii) Virus transfer from fingerpads to disks. Nearly 25% of the input virus PFU remained detectable on the fingerpads even after 4 h (Fig. 4). The amount of virus transferred from contaminated fingerpads to clean disks on contact was nearly 27% of the virus (3,484 PFU) remaining after 20 min of drying; when the inoculum was dried for 4 h, 1.6% of the surviving virus (50 PFU) was transferred.

(iii) Virus transfer between fingerpads. About 10,433 PFU of HAV was deposited on the fingerpad of volunteer E, but only 1,967 PFU (19%) remained at the end of 4 h (Fig. 5). The survival pattern was similar to that observed earlier (Fig. 1). The amount of HAV transferred from the fingerpads of volunteer E to the fingerpads of volunteer A was 2,800 PFU after 20 min of drying (23% of the available PFU) but only 17 PFU (0.9% of the available PFU) at the end of 4 h.

DISCUSSION

Gwaltney et al. (14) demonstrated that as much as 70% of the infectious rhinovirus on hands can be transferred to a recipient finger after a contact of only 10 s. Similarly, Hendley et al. (20) have shown that dried rhinoviruses picked up from environmental surfaces may persist on human hands long enough to permit self-inoculation and that virus transmission may proceed by transfer of the virus from the hands of the infected person to an intermediary surface or directly to the fingers of a susceptible recipient. Respiratory syncytial virus has also been shown to survive in the environment, allowing its transfer to human hands (18), and studies on the transfer of

human rotavirus between hands and inanimate surfaces also implicate hands and inanimate surfaces as potential rotavirus vehicles (2). The results of all of these studies suggest that human hands and environmental surfaces may be potential routes of virus transmission.

The results presented here show that considerable amount of HAV remained infectious on the fingerpads after 4 h, even though 68% of virus infectivity was lost in the first 1 h. Whereas the reasons for the biphasic pattern of virus decay and the relatively rapid drop in virus titer during the first 60 min are not clear, it may be related, at least in part, to the rate of moisture loss from the inoculum. Although HAV inocula appeared dry after about 20 min, the decay curve shown in Fig. 1 leveled off at around 60 min. This suggests that 60 min was the end of the drying period.

Other factors, such as the physiology and chemistry of the skin surface, may also play a role in HAV survival and inactivation on hands. Both immunoglobulins and serum proteins contained in normal human sweat have been shown to inhibit bacterial growth (38). It is not clear that these substances behave in a similar way with viruses. Surface immunoglobulin A has also been shown to contribute to the immunological defense of the skin (23). The presence of two HAV subpopulations in the inocula may also be responsible for the biphasic pattern in virus decay, so that the more susceptible virus population is inactivated faster during the drying period and the more resistant virus population persists over the whole study period (4 h).

Because a fecal suspension of HAV was used to simulate natural conditions in which HAV contaminates both animate and inanimate surfaces, it is expected that the results may be an indication of what may occur in the field. Residual HAV on human hands at the end of 4 h could also be transferred to others and may be enough to initiate infection upon self-inoculation. High levels of infectious HAV were exchanged between the fingerpads and the disks over the 4-h period. These results suggest that human hands and fomites are potential ways in which self-inoculation and communication of the virus can occur over an extended time. The significance of pressure and friction in determining the amount of HAV transferred in the three models examined in this study was shown. We are not aware of any previous quantitative studies in which such an interaction in the transfer of infectious virus has been documented.

Virus transfer between contaminated and clean surfaces is greatest when the inoculum is wet (2, 14, 18, 20) and is observed to decrease as the virus dries. Also, during drying some virus is usually inactivated. In the observed transfer of infectious virus at specific times, the effects of drying on virus inactivation as well as the physical transfer of virus particles and their recovery are confounded. However, in order to assess the virus loss because of drying, we used a reference point, which was the indication that the virus inocula were visibly dry on the contaminated surfaces. An inoculum of 10 μl of HAV in fecal suspension appeared to be dry after 18 to 20 min on the metal disks (33) and 11 to 17 min on fingerpads. For this reason, 20 min was taken as the minimum time that HAV inocula would require to initially become dry. The first

reading was therefore taken at 20 min and indicates virus loss because of drying.

The period of experimentation used in this study is within the limits for human hands to stay contaminated before a hand wash or interaction with other animate and inanimate environments. The metal disks used in this study were selected to represent environmental surfaces. In our previous studies (33), stainless steel disks allowed the virus to persist for several days; glass and plastic surfaces may behave similarly (44).

HAV outbreaks are often associated with hospitals (3, 13, 25, 45). The mode of transmission in those studies was unclear, but the source was usually an asymptomatic child or adult. The observation that the spread of the infection is associated with nurses and other medical personnel suggests that human hands may play a vehicular role in virus transmission.

An assessment of HAV outbreaks in the community shows that diaper-changing personnel in many day-care centers also participate in food-handling activities (9, 16); HAV may be acquired from children who are excreting HAV; the majority of these children are asymptomatic (10). Although no such study has been done for HAV, the day-care center environment has often been found to be contaminated with enteric organisms which have modes of transmission similar to those of HAV (9, 24, 39).

HAV that has contaminated toys and other play items may persist for prolonged periods of time (33); toys are shared items in day-care centers, and children are known to suck them frequently (4, 9). Such behavior tends to encourage virus transmission through the fecal-oral route

among the children. The children also associate closely with the environment around them as well as with adults assigned to them in the day-care center (4). Interactions between such a contaminated environment and the institutionalized population, especially children, may recycle the virus until susceptible hosts, usually adults, are infected. Such a secondary spread of HAV often results in communitywide outbreaks (17).

Restaurant-associated HAV outbreaks are frequently reported (7, 26, 28, 35). In these instances, the index case was frequently a food handler who was subclinically infected with HAV. Secondary spread of the disease is a prominent feature of the outbreaks (7, 26, 28) and may occur over an extended period of time (30), indicating a continuing source of infection. Refrigerated foodstuffs as well as foods stored at room temperature after handling are know to permit the survival of HAV for several days (11) and, together with the index case, furnish a continued source of the virus. It has also been suggested that HAV which persisted on a sandwich board contaminated by hands moistened with oropharyngeal secretions from an anicteric food handler was responsible for contaminating many sandwiches (28).

The potential of human hands to spread viral agents has serious implications in the food industry and eating establishments, and it may be particularly important when foodstuffs do not require cooking after handling. HAV contamination of foodstuffs by an institutional food handler can culminate in communitywide HAV outbreaks (6, 7, 19, 26, 28, 35).

The persistence of HAV on surfaces and the ability of the virus to be interchanged in animate and inanimate environments suggests that human hands and environmental surfaces constitute important epidemiological factors in the spread of HAV. Approximately 50% of HAV cases reported to the Centers for Disease Control every year show no identifiable source of the virus (15). Previously, we have shown that HAV on environmental surfaces can remain infectious for several days (33). Taken together with the results of this study, this suggests that human hands and environmental surfaces may serve as sources of HAV dissemination, especially in institutionalized populations.

Hand washing is probably the single most important measure for controlling and preventing the spread of infections (5, 22, 43). However, compliance with hand washing guidelines among medical personnel is frequently suboptimal (1, 10) and is sometimes ignored altogether (9). Moreover, many commonly available surface disinfectants have been shown to be ineffective against HAV (29), and Mbithi et al. (34) have shown that commonly available antiseptics are inefficient in removing or inactivating HAV. Use of antiseptics has previously failed to contain nosocomial HAV (3, 45), thus permitting secondary spread of the virus in hospital wards.

The findings of this study show that the control and prevention of HAV outbreaks in settings such as day-care centers, hospitals, eating establishments, and other, similar facilities must place a great emphasis on both human hands and environmental surfaces as virus vehicles.

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TRANSMISSION OF EXPERIMENTAL RHINOVIRUS INFECTION BY CONTAMINATED SURFACES

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Gwaltney, J.M., Jr. (Dept. of Internal Medicine, U. of Virginia School of Medicine, Charlottesville, VA 22908) and J. O. Hendley. Transmission of experimental rhinovirus infection by contaminated surfaces. *Am J Epidemiol* 1982;116:828-33.

Transfer of experimental rhinovirus infection by an intermediary environmental surface was examined in healthy young adults, in four studies done in 1980-1981, by having recipients handle surfaces previously contaminated by infected donors. Recipients touched their nasal and conjunctival mucosa after touching the surfaces. Five (50%) of 10 recipients developed infection after exposure to virus-contaminated coffee cup handles and nine (56%) of 16 became infected after exposure to contaminated plastic tiles. Spraying of contaminated tiles with a commercially available phenol/alcohol disinfectant reduced (p=0.003) the rate of recovery of virus from the tiles from 42% (20/47) to 8% (2/26). Similarly, the rate of detection of virus on fingers touching the tiles was reduced (p=0.001) from 61% (28/46) with unsprayed tiles to 21% (11/53) with sprayed tiles. Fifty-six percent (9/16) of the recipients exposed on three consecutive days to untreated tiles became infected while 35% (7/20) touching only sprayed tiles became infected with rhinovirus (p=0.3). These studies indicate

that experimental rhinovirus colds can be spread by way of contaminated environmental surfaces and suggest that disinfectant treatment of such surfaces may reduce risk of viral transmission by this route.

acute respiratory disease viruses; disinfectants; rhinoviruses

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Rhinovirus may infect susceptible persons by means of self-inoculation of virus which accidentally contaminates the fingertips (1-3). It is clear that virus on the fingers of volunteers is efficiently transferred by contact to the nasal and conjunctival mucosa where it routinely produces infection (2). An unsettled question is how virus might get onto the fingertips of well persons. The two most likely sources are skin of persons with colds and objects in the environment which have been contaminated with virus by ill people.

Rhinovirus has been routinely recovered from the hands of persons with both natural (1) and experimental (2, 4, 5) infections. Also, rhinovirus was detected on 6 percent of selected objects in the homes of persons with rhinovirus colds (2). Thus, measures which would eliminate or reduce viral contamination of skin and environmental surfaces may be useful in interrupting viral spread and controlling rhinovirus colds.

Hand treatment with a virucidal preparation was successful in interrupting rhinovirus transmission between volunteers (6), and its effectivenesses is now being studied under natural conditions. The current study was done to determine if rhinovirus colds could be efficiently transmitted in volunteers by way of contaminated surfaces. In

addition, the effectiveness of a commercial disinfectant in killing virus on surfaces and interrupting transmission at that point was assessed. Surfaces contaminated with virus by volunteers with experimental colds were sprayed with phenol/alcohol disinfectant or left unsprayed to determine the efficacy of this form of disinfection in interrupting spread of infection to volunteers touching the surfaces.

MATERIALS AND METHODS

Volunteers

Healthy, young adults (≥18 years) were recruited from the University of Virginia community, Charlottesville, VA, in four studies done in 1980-1981. Volunteers were selected if serum-neutralizing antibody titers to the challenge virus were low or absent (≤2). Volunteers who had symptoms of a respiratory illness in the week prior to the study were excluded. Women who were pregnant or not using accepted contraceptive methods were excluded. The experimental protocol was approved by the University of Virginia Human Investigation Committee and volunteers gave written consent to participate in the study.

Virus challenge pool

A pool of a presently unnumbered rhinovirus strain (HH) in nasal secretions from a young adult with a rhinovirus cold was used for the challenge inoculum. Nasal secretions were diluted 1:5 in Hanks' balanced salt solution before storage in multiple aliquots at -70 C. The titer of the pool was 10^3 tissue culture infective $dose_{50}$ ($TCID_{50}$)/

ml. The pool had been tested for and found free of other viruses, pathogenic bacteria, nycoplasmas, fungi, and *Mycobacterium tuberculosis*. The individual from whom the pool was obtained was otherwise in good health and had a negative chest x-ray, tuberculin skin test, and serum test for hepatitis B virus.

Specimen collection and testing

Samples of nasal and pharyngeal secretions were obtained by cotton swabs which were placed in 5 ml of beef heart infusion broth with 1 per cent bovine serum albumin and antibiotics. Fingers were rinsed with 1.5 ml of the same broth without antibiotics, and the rinse was combined with 1 ml of broth with antibiotics before testing. The plastic and ceramic surfaces were cultured by rinsing with 2.5 ml of the collecting broth with antibiotics.

Viral culture was performed by standard methods (7) in human embryonic lung cells (WI-38) and/or fetal tonsil cells in screw-capped tubes. Serum specimens were obtained before and three weeks after challenge. Serum-neutralizing antibody titers were determined against the challenge virus by a standard method (7).

Infectivity and illness criteria

Infection was judged to have occurred if virus was recovered from a nose/throat culture an/or if there was a fourfold or greater serum neutralizing antibody rise to that challenge virus. Determination of the occurrence of a symptomatic illness was based on a modification of the

method of Jackson et al. (8) Illness was considered present if a volunteer had a minimum symptom score of six and had the subjective impression that he had a cold, or if rhinorrhea was present on three or more days of observation.

Disinfectant

A commercial spray disinfectant (Lysol spray disinfectant, Lehn and Fink Products Group, Sterling Drug Inc., Montvale, NJ) containing the active ingredients O-phenylphenol (0.1 percent), N-alkyl (C_{18}, 92 percent; C_{16}, 8 percent), N-ethyl morpholinium ethylsulfates (0.035 percent), and alcohol (67.748 percent) was dispensed from an aerosol can as spray.

Experimental plan

Infection of donors. Immediately following a nasal wash with normal saline (5 ml/nostril), donors were inoculated intranasally with 0.25 ml per nostril of a $10^{0.5}$ dilution of the virus pool. Challenge was done on two successive days. Donors were placed in isolation 12 hours after the second inoculation (the earliest that infected donors would be expected to shed virus). They were monitored on three exposure days (days 3, 4, and 5 after the first challenge) for the amount of virus in their nasal secretions and on their hands (after they had contact with the contaminated surface). Each donor was lodged in an individual room and separated from the recipients at all times.

Donor handling of surfaces. Symptomatic donors deliberately contaminated their hands as in nose blowing and nose wiping immediately after contact with a ceramic coffee cup or a 12.5 x 12.5 cm square plastic tile of the type used for counter tops. The donors then held the handle of the coffee cup or rubbed the tile with the fingertips of each hand for approximately five seconds. This was repeated on three consecutive exposure days, beginning on the third day after the donor's first inoculation. The same donor was not necessarily paired with a particular recipient on each of the three exposures days.

Treatment of tiles. Ten minutes after the donors handled the surfaces of the tiles, the tiles were sprayed with disinfectant or not. The dispenser was held 15-20 cm from the surface which was sprayed for two to three seconds until covered with mist.

Recipient handling of contaminated objects. For exposure to the coffee cups, the recipients held and rubbed the handle of the cups as if drinking. For exposure to the tiles, the recipients touched and rubbed the surfaces with the fingertips. This was done 10 minutes after the spray treatment to allow time for treated tiles to dry. Fingertips of each hand touched a different tile (of the two contaminated by the donor). After contact with coffee cup or tiles, the recipient placed the fingers in contact with the conjunctival and nasal mucosa as in rubbing the eyes and probing the nose. The recipient's fingers and coffee cup handles or tiles were then sampled for virus. Recipients were housed in isolation in individual rooms beginning 12 hours after the second contact.

RESULTS

Exposure by coffee cup handles. Three donors were used to contaminated coffee cup handles. Five to the 10 recipients exposed to the coffee cup handles became infected. Virus was recovered in nose/throat specimens from all five infected recipients on two or more days, and all had fourfold or greater serum-neutralizing antibody rises to the challenge virus. Virus was recovered from one coffee cup handle specimen and from hand rinse specimens of two of the infected recipients.

Exposure to plastic tiles. In four experiments, recipients contacted tiles which had been contaminated by donors and either sprayed with disinfectant or left unsprayed (table 1). Virus was detected on 20 (42.5 percent) of 47 tiles that received no treatment compared to 2 (8 percent) of 26 which had been sprayed with disinfectant ($p= 0.003$). Virus was detected in 28 (61 percent) of 46 hand rinses of recipients in the no treatment group and 11 (21 percent) of 53 hand rinses of recipients in the disinfectant spray group ($p=0.001$). Nine (56 percent) of 16 recipients who contacted tiles which had not been sprayed became infected. Seven (35 percent) of 20 recipients became infected after touching tiles which had been sprayed with disinfectant ($p=0.3$). All infected recipients had virus in their nose/throat cultures on one or more days, and 13 of 16 had fourfold or greater rises of serum-neutralizing antibody titer to the challenge virus.

Results in both the treatment and no treatment groups were combined and analyzed to determine whether the presence of detectable virus on the tiles correlated with viral presence on the recipients' fingers and whether the

virus on the recipients' fingers correlated with the development of infection. In 15 (75 percent) of the 20 instances in which tile cultures were virus-positive, fingers of recipients contacting the tiles were positive for virus. In contrast, only 18 (36 percent) of the 50 instances in which tile cultures were negative were the matching hand cultures from recipients positive ($p=0.007$, Fisher's exact test). Virus was detected on the hands of 23 recipients at least once during the three exposure days. Fourteen (61 percent) of these 23 individuals were infected, whereas only 2 (15 percent) of 13 recipients whose hand cultures were negative became infected ($p=0.02$, Fisher's exact test). Thus, it appeared that the presence of detectable virus was a controlling variable both in viral transfer from tile to hands and from hands to susceptible mucosa.

Clinical illness in recipients

Of the 16 recipients who became infected, 10 (62.5 percent) met the criteria for illness. Symptom scores of these individuals ranged from 8 to 42 with a median score of 23. The illness had the characteristics of a typical rhinovirus cold.

DISCUSSION

In this study, experimental rhinovirus infection was transmitted by two types of contaminated surfaces, ceramic coffee cup handles and plastic tiles. The efficiency of transmission by the two surfaces was similar, 50 percent for the coffee cups and 56 percent for the tiles. Treatment of the tiles with a phenol/alcohol spray disinfectant was effective in reducing viral contamination of

the tiles and viral soiling of fingers having contact with the tiles. Further, the infection rate of susceptible recipients contacting the tiles may have been reduced by the disinfectant treatment, although the difference was not statistically significant. Since we found that the risk of acquiring infection was directly related to the presence of viral contamination of the fingers and this, in turn, was related to the presence of virus on the tiles, it could reasonably be expected that the use of a disinfectant which would eradicate virus on surfaces would reduce the infection rate by this route. In view of this, it was felt that further use of volunteers to investigate this question was not indicated.

The question of whether disinfection of environmental surfaces would result in reduced transmission of rhinovirus colds under natural conditions cannot be answered until the importance of the hand contamination/self-inoculation route of spread is known. At present, the evidence with experimental colds suggest that the hand/self-inoculation route is likely to be important under natural conditions (2,3), but direct evidence bearing on this question has not been obtained. The disinfectant tested in these experiments was moderately effective in eliminating rhinovirus from a surface and has proven to be safe through long use. However, it wasn't ideal in that it did not completely eliminate viral contamination of the tiles and one-third of the recipients in the tile-treated group became infected. If the hand/self-inoculation route does prove to be important, environmental disinfection may have a useful role in controlling colds.

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REFERENCE NO. 13

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Letter to the Editor

Inactivation of Human Immunodeficiency Virus-1 at Short Time Intervals Using Undiluted Bleach

To the Editor: Several modes of transmission are implicated in the spread of human immunodeficiency virus-1 (HIV-1) and acquired immunodeficiency syndrome (AIDS) including injection practices, blood transfusion, sexual activity, and perinatal transmission. Injection drug users are at risk for HIV-1 transmission due to sharing needles (1-3). Health care workers and laboratory personnel are at risk for HIV-1 infection through accidental needle stick injuries and exposure of their mucous membranes to HIV-1 contaminated fluids while providing treatment to infected patients or processing fluids and tissues from these individuals (4).

The use of hypochlorite-based household bleach has been widely recommended to health care workers and laboratory personnel as a means to inactivate HIV-1 (5,6) and injection drug users have been advised to clean their injection equipment with household bleach (7-9).

Prior studies have characterized the use of hypochlorite and other reagents to inactivate HIV-1 (10,11). Our investigation provides additional detailed information regarding the shortest exposure time required to inactivate HIV-1 with undiluted household bleach.

METHODS

Normal peripheral blood mononuclear cells (PBMNCs), cells infected with HIV-1, and supernatant stocks of HIV-1 were prepared according to standard methods (12,14). HIV p24 antigen was detected utilizing kits from Abbott Laboratories (Chicago, IL. U.S.A), according to manufacturer instructions. The p24 assay is generally acknowledged to be more sensitive than reverse transcriptase assays (15). Household bleach was purchased from a local supermarket and contained 5.25% sodium hypochlorite.

HIV-1 was pelleted from infected cell culture supernatants at 13,000 relative centrifugal force (rcf) (12) in microcentrifuge tubes. The pellets were then exposed to bleach for various periods of time, ranging from 15 s to 5 min. After gently removing the bleach from the tubes, the pellets were suspended immediately in cell culture medium and used to infect normal donor PBMNCs using standard techniques (13,14). The carryover of bleach into the culture medium from pelleted virus was approximately 0.2% (v/v), which we determined to be completely nontoxic for PBMNC cultures. Toxicity only occurs when the bleach concentration is above 1% (v/v) (data not shown). The PBMNC cultures were maintained for up to 28 days and were assayed for virus production at weekly intervals by determination of HIV-1 p24 is the culture medium. Cultures were classified as negative if all determinations of p24 production were below 5 pg/ml for that culture. A culture was determined to be positive when p24 production was three times above the maximum background value of 5pg/ml. The peak mean value was reported for these cultures and generally occurred

after 14 or 21 days of cell culture. All experiments were done in triplicate, and exposures of virus to bleach for less than 1 min were repeated in triplicate two times.

The viability of normal PBMNCs was determined after treatment with bleach by typan blue dye exclusion and taking counts using hemacytometer. Aliquots of 10 million cells were pelleted by centrifugation at 900 rcf and suspended in 0.5 ml of undiluted bleach. After exposure to bleach ro 15 to 60 s the suspensions of cells were immediately diluted to 50 ml (100-fold) using RPMI medium containing 500 units/ml each of penicillin and streptomycin, and 1% of fetal calf serum. A 1% dilution of bleach and lower concentrations was completely harmless for cell viability as stated above. The cells were then pelleted as above and suspended in 0.5 ml of RPMI medium supplemented with antibiotics and 20% fetal calf serum. Cell viability was determined in duplicate for each time point up to 60 s and after maintenance in cell culture medium for 18 h.

RESULTS

HIV-1 was completely and consistently inactivated by undiluted household bleach at all tested exposures of 30 s or longer. As summarized in Table 1, HIV-1 was not completely inactivated at shorter intervals or exposure to bleach. The p24 antigen concentrations reported in Table 1 are averages for triplicate experiments at the indicated bleach exposure time periods. HIV-1 was not consistently inactivated at exposures to undiluted bleach of 15 and 25 s. Exposure of HIV-1 to a 10% dilution of bleach, using

the same methodology, did not inactivate the virus even at 5 min of exposure.

PBMNCs were also shown to be rapidly fragmented and killed in the presence of undiluted bleach. There was complete loss of viability at 25 but not at 20 s of exposure to undiluted bleach (Table 2). With 10% diluted bleach, 4% of the PBMNCs remained viable after 60 s. After 18 h in cell culture medium, 3% of the cells exposed to 10% diluted bleach remained viable.

DISCUSSION

Our present investigation gives a precise measurement of bleach exposure time for the inactivation of HIV-1. The methods used demonstrate that exposure of pelleted virus to undiluted household bleach inactivates HIV-1 and kills PBMNCs within a short exposure period, but a 10% dilution of bleach is ineffective at considerably longer exposure periods. We found that the 10% dilution had viable virus at 5 min of exposure. Based on these results, 10% diluted bleach does not appear to have any practical, effective value in the inactivation of HIV-1.

There are clear implications from these findings: To inactivate HIV-1, use undiluted bleach for a minimum exposure of 30s. It is also important to note that PBMNCs were killed after 25 s of exposure to bleach.

It is extremely important to make this information available to all persons at potential risk of exposure to HIV-1, including health care and research workers as well as injecting drug users. This is crucial as part of national and international efforts to halt the spread of AIDS.

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REFERENCE NO. 14

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Thirty-nine products representing six categories of disinfectants (alcohols, chlorines, dilute glutaraldehydes, iodophors, phenolics, and quaternary ammonium compounds) were first tested in the absence of bioburden, using four test methods with five test organisms. Products that performed best were retested with the same methods and organisms in the presence of both serum and whole blood, using 3- and 10-minute contact times. Only products containing high ethyl alcohol had consistently high antimicrobial activity regardless of the test method, test organism, or contact time used both in the absence and presence of bioburden. Although these specific formulations demonstrated ability to penetrate and inactivate high concentrations of microorganisms within heavy bioburden, optimum disinfection of environmental surfaces is highly formulation dependent. Other products tested showed deficiencies that contraindicate their use as environmental surface disinfectants in clinical dental settings.

Antimicrobial activity of environmental surface disinfectants in the absence and presence of bioburden

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Current methods of dental treatment cause widespread contamination of the operating area.[1-8] Droplet

splatter, flying debris, contaminated hands and instruments, and operation of automated instruments such as handpieces, ultrasonic scalers, and air-slurry polishers contribute to the problem.[1-3,9-20]

The potential of contaminated environmental surfaces to transmit infection continues to be questioned by some investigators. However, confirmation of long-term survival of large numbers of pathogenic organisms on a variety of surfaces[21-35] has made infection via contaminated surfaces credible, and experimental data corroborate the possibility. For example, both rotavirus and rhinovirus have been transferred from contaminated hands, to objects, and to clean hands in sufficient numbers to elicit infection in susceptible humans.[36-37]

Furthermore, colds caused by rhinovirus have been transmitted to human volunteers experimentally via contaminated ceramic cup handles and plastic tiles.[38] Comparable correlations at the clinical level have been difficult because so much time elapses between infection and overt symptoms that subjects often cannot recall objects contacted. Even when recall is possible, the incubation time lapse can exceed organism survival time on inanimate surfaces, and culturing becomes impossible. Therefore, clinical reports linking infection with objects have been based on circumstantial evidence. Examples include epidemiologically linked diseases,[21,39-44] spread of infection in facilities in which direct contact of infected subjects was impossible,[45-46] clinical experiments,[47] and documentaries.[48] Reports such as these make it impossible to rule out environmental surfaces as fomites.

Until data become available to demonstrate conclusively that contaminated environmental surfaces cannot transmit infections, clinicians cannot ignore or treat them lightly. As dental operatories have innumerable environmental surfaces that are contaminated during routine patient treatment, the effectiveness of products used to disinfect these surfaces must be examined. This investigation was conducted to test a number of commercial products to identify those with broad-spectrum, rapid antimicrobial activity both in the absence and presence of bioburden.

Methods and materials

The general protocol specified use of four test methods and five test organisms with 39 disinfectants in the absence of bioburden. Disinfectants demonstrating best antimicrobial activity under these conditions were then tested with the same methods and organisms in the presence of bioburden.

Disinfectant selection and preparation

Table 1 lists the disinfectants selected for this study based on a product-use survey,[49] manufacturers' communications, and literature review. All products were prepared according to manufacturers' directions immediately before testing. When dilution was specified, sterile deionized water was used.

Test organism selection and preparation

Test organisms were: *Pseudomonas aeruginosa* ATCC 15442, *Salmonella choleraesuis* ATCC 10708, *Staphylococcus aureus* ATCC 6538, *Mycobacterium bovis* (BCG) ATCC 35743, and poliovirus type I (Mahoney strain). The four bacteria were selected because they are specified as test organisms by the Environmental Protection Agency (EPA) to register hospital disinfectants[50] and to establish tuberculocidal claims.[51] Poliovirus I was selected because it is resistant to inactivation by many disinfectants.[52-54]

Bacteria were prepared according to EPA specifications, except stock cultures were stored in liquid nitrogen. Poliovirus was grown in HeLa cells, harvested by multiple freeze/thaw cycles and cesium chloride (CsCl) banding, and stored at 4 C in CsCl.

Test methods

Association of Official Analytical Chemists Use Dilution Method (AOAC UDM). The standard AOAC UDM, using 60 stainless steel penicylinders per replicate, was performed precisely as described in AOAC literature.[50] Secondary subculture was performed on each carrier and both subcultures were incubated at 37 C for 48 hours. For bioburden testing, the same method was used except penicylinders were coated with 50:50 vol/vol mixture of human whole blood and bacterial culture.

Environmental Protection Agency Tuberculocidal Activity Test Method (EPA TB ATM). The standard quantitative EPA TB ATM was performed precisely as specified in EPA literature.[51] For bioburden testing, the same method was

used except test suspensions contained 50% horse serum which delivered a 5% concentration of horse serum to the disinfectant.

Virucidal Suspension Test. A suspension test, rather than a carrier method, was used for all virucidal testing because all attempted procedures for drying virus onto carrier surfaces, and subsequent virus recovery, caused loss of viral titer that was unpredictable and unacceptably high (>2 $\log_{10}$ units). Tests performed without blood used 100 µL of virus, $\geq 10^6$ plaque-forming units (PFU), added to 900 µL of disinfectant. After prescribed contact times, phosphate-buffered saline solution was used for serial 1:10 dilutions, and four consecutive dilutions of virus were assayed in duplicate on monolayers of HeLa cells for infectious poliovirus. Twelve-well plates (12-Well Tissue Culture Cluster 3512, Costar) were incubated at 37 C for 48 hours and stained. Log_{10} reductions were calculated from plaque counts. Disinfectants yielding three $\log_{10}$ reductions in virus titer were evaluated for interference with virus attachment to HeLa cells. Each 12-well plate included controls to assay for titer of viral challenge and test for system contaminants. Also, disinfectant cytotoxicity to HeLa cells was determined. In cases in which cytotoxicity was detected, dilution was used to eliminate this effect. Dilution was also used to eliminate the effect of residual disinfectant on the virus in the assay system.

Testing with human whole blood used the same procedures described except 10µL of poliovirus ($\geq 10^6$ PFU) was added to 100 µL of blood and allowed to stand for 1 minute before 900 µL of disinfectant was added. Controls were included to determine amount of virus inactivated by blood. For tests in which urea was included to disrupt

ethyl alcohol-induced blood aggregates, the same procedures were employed except that siliconized tubes (Sigmacote, Sigma Chemical) were used and 9.0 mL of 7.0 mol/L urea was added to the blood-virus-disinfectant mixture after prescribed contact times. Log_{10} reductions were calculated for all work involving poliovirus (both with and without bioburden) using the formula: log_{10} reduction = log_{10} (titer of viral challenge per mL) – log_{10} (titer infectious virus per mL after exposure to disinfectant).

Clinical Research Associates Environmental Wipe Method (CRA EWM). A test was devised to mimic dental clinical procedures used for disinfection of environmental surfaces to determine if wiping with disinfectant-soaked gauze sponges inactivated organisms dried onto surfaces, both in the absence and presence of bioburden. Silicone adhesive (Mirror 3 Tray Adhesive, Kerr/Sybron) and caulking (Silicone II GE5070, General Electric Co) were used to attach an $8^{1/16-}$ x $11^{7/8-}$ in piece of laminated plastic counter covering (1595-6 Black Wilsonart, Ralph Wilson Plastic Co) to $8^{1/4-}$ x 12-inch polypropylene trays (Size B Trays 20Z401, Zirc Dental), which were trimmed to fit snugly under their polyethylene lids (Tray Cover 20Z441, Zirc Dental). Figure 1 shows the test tray after construction and before inoculation with test organisms. Lids and trays were sterilized with ethylene oxide (ETO) for 2 hours at 135 F and aerated for 8 hours before 2 mL of bacterial suspension was applied with a sterile 2- x 2-in cotton filled gauze sponge (Cotton Filled Sponges 6000207, Healthco). After the organisms applied to the trays were completely dried (20-30 minutes) in a laminar

flow hood (NU-408FM-600, Nuaire), 3.5 mL of disinfectant was pipetted onto a sterile gauze sponge which was then used to wipe the test surface for 10 seconds using about 150-g pressure with overlapping strokes (20 left to right, followed by 20 top to bottom). Disinfectants sold in aerosol spray cans were treated in the same manner to standardize the amount of disinfectant delivered to the contaminated surface by spraying the disinfectant into a sterile test tube before pipetting. After wiping, the disinfectants were left on the trays for 3 minutes before one of two methods was used to determine the number of viable organisms remaining on the tray. The method depended on the test organism.

M bovis coated trays were flooded with 50 mL of tryptic soy broth (TSB) with neutralizers (ingredients are enumerated in section on media and neutralizers), and scrubbed for 1 minute with a sterile polypropylene brush (Nail Brush 501, Kellogg Brush Co) to remove and suspend viable organisms. The fluid was collected and diluted, and duplicate 1-mL samples of each dilution, plus the undiluted fluid (approximately 40 mL), were passed through a 0.45-µm filters. Each filter was washed twice with 100 mL of sterile 0.1% peptone water, placed on Mycobacterium 7Hll agar, and incubated at 37 C for 21 days. To allow calculation of $\log_{10}$ reductions, trays wiped with water were included in all tests. Log_{10} reductions were calculated using the formula: $\log_{10}$ reduction = $\log_{10}$ (number of viable organisms from water control tray) – $\log_{10}$ (number of viable organisms from test tray).

P aeruginosa, S choleraesuis, and *S aureus* remaining viable organisms were assayed directly on the trays by adding 300 mL of tryptic soy agar (TSA) with neutralizers

at 45 C. Trays were covered with their lids and incubated at 37 C for 48 hours. Colonies were counted, with > 500 designated as "too numerous to count" (TNC). Trays wiped with water were included as organism viability controls. Results of this testing were reported two ways: by the number of colony forming units (CFU) for each of the three test bacteria that survived disinfectant treatment, and as percent of tests less than TNC. To determine the percent of tests less than TNC for a particular disinfectant, the following formula was used: percent of tests less than TNC = number of tests less than TNC for the three organisms ÷ total number of tests x 100. To determine the mean percent of tests less than TNC for a category of disinfectants with the same active ingredient, the mean of all disinfectants within the category was calculated.

The CRA EWM bioburden testing with all four bacteria used the same methods, except cultures were mixed 50:50 vol/vol with human whole blood; 1 mL of this mixture was spread on trays with a sterile glass rod. For tests with *S aureus* and *M bovis* in which urea was included to disrupt blood aggregates, the procedures described previously for *M bovis* were used except that 50 mL of 7.0 mol/L urea was substituted for TSB used to suspend organisms from the surface.

Media and neutralizers

For *M bovis*, TSB (Difco) containing 1% Tween 80 (Fisher Scientific), 1% lecithin (Sigma), and 0.4% sodium thiosulfate (Sigma) were used in CRA EWM tests, and Mycobacterium 7H11 agar (Difco) was used for all subcultures.

For *P aeruginosa, S choleraesuis,* and *S aureus,* TSA (TSB + 1.5% Bacto-agar, Difco) containing 0.5% Tween 80, 0.1% lecithin, and 0.1% sodium thiosulfate was used as the subculture medium. Minimum Essential Medium (Irvine Scientific) supplemented with 10% calf bovine serum enriched with iron (HyClone) was used for HeLa cell cultures.

Selection of bioburden and procedures used for testing with bioburden

Because of its clinical relevance, human whole blood was used as the bioburden challenge whenever possible. A concentration of 50% human whole blood in the blood-bacterial suspension mixture was used with the AOAC UDM and CRA EWM tests performed with *P aeruginosa, S choleraesuis,* and *S aureus.* A 10% concentration of human whole blood was used with virus suspension tests because higher concentrations precluded proper mixing and pipetting with some disinfectants. Two different types of bioburden were used with *M bovis* to accommodate test method differences. A 50% concentration of human whole blood in the bacterial suspension was used in the CRA EWM tests with this organism, and 5% horse serum in the bacterial suspension-disinfectant mixture was used in the EPA TB ATM tests. Five percent horse serum was used because it has been specified as the bioburden for other standard EPA tests.[55]

Use of human whole blood as the bioburden required special procedures when ethyl alcohol-containing disinfectants were used because they caused the organism-media-whole blood mixture to form aggregates. Different

chemicals were sought to disperse the aggregates without affecting viable organisms, and 7.0 mol/L urea met the criteria best.[56] Although 7.0mol/L urea destroyed gram-negative bacteria, it allowed almost complete recovery of *M bovis, S aureus,* and poliovirus. Therefore, when ethyl alcohol disinfectants were tested with whole blood bioburden, the CRA EWM tests using *S aureus* and *M bovis* and suspension tests using poliovirus included the addition of 50mL and 9mL of 7.0 mol/L urea, respectively, after disinfectant treatment. The same tests were performed without urea so results could be compared.

Selection of wipe material

As it has been reported that cotton may interfere with the antimicrobial activity of iodophors,[8] this was also evaluated. For this test, sterile GSA centrifuge bottles containing 64 mL each of Biocide and Wescodyne iodophors diluted 1:213 received 8 g of three different wipe materials (Cotton-Filled Gauze Sponges by Healthco, Nu-Gauze rayon/polyester sponges by Johnson and Johnson, and Viva Paper Towels by Scott Paper Co.) After 10 minutes, samples were centrifuged for 20 minutes at 6,000 x g. An aliquot of 9.9 mL was removed from each GSA bottle and 0.1 mL of *S aureus* culture was added. Assays for viable organisms were performed at 3 minutes.

Tests for chemical interference and neutralizer efficacy

To assay for possible toxic effects of ETO residuals or materials, or both, used in the CRA EWM, 300 mL of TSA with neutralizers containing about 100 CFU was poured

into an ETO sterilized CRA EWM tray, a CRA EWM tray disinfected with 70% vol/vol denatured ethyl alcohol and not ETO sterilized previously, and several large sterile petri plates (150 x 15 mm). Tests used three replications each of *P aeruginosa, S choleraesuis,* and *S aureus.*

To test efficacy of the neutralizers in TSB, 1 mL of each disinfectant was added to 9 mL of TSB with neutralizers. After 1 minute, about 100 CFU of *M bovis* was added. Thirty minutes later, the suspension was filtered and subcultured on Mycobacterium 7H11 agar. Efficacy of the neutralizers used in TSA was evaluated by wiping CRA EWM trays with disinfectant, waiting 3 minutes, then filling the trays with TSA plus neutralizers containing about 100 CFU.

Reproducibility of the wipe test

To determine reproducibility of the mean organism challenges applied to CRA EWM test trays both without and with blood, results from water control trays were examined. Reproducibility of CRA EWM test procedures was examined when two different technicians performed the test with *M bovis* mixed 50:50 vol/vol with human whole blood; diluted Clorox (1:5) was the disinfectant. Tests were performed in parallel, and the technicians alternated tray treatment to correct as much as possible for time.

Results

Comparison of data from four different test methods

Figure 2 includes the data from all tests performed in the absence of bioburden. Disinfectants are grouped by main active ingredient to condense the data to facilitate comparison of results obtained with the four different test methods (AOAC UDM, EPA TB ATM, CRA EWM, and suspension tests). The data show a close correlation of results obtained with the EPA-specified test methods (EPA TB ATM and AOAC UDM) compared with the wipe test method (CRA EWM). Significant differences were evident only with iodophors tested against *M bovis*. With this particular disinfectant-test organism combination, the suspension test method (EPA TB ATM) was significantly more permissive than the surface wipe test method (CRA EWM).

Antimicrobial activity in the absence of bioburden

Table 2 lists the detailed data by CFU and PFU under each of the five test organisms for all 39 disinfectants tested in the absence of bioburden. When these data were combined under the primary active ingredient of each disinfectant (Fig 2), it became apparent that overall, in the absence of bioburden, ethyl alcohols and chlorines provided best inactivation of all five test organisms, regardless of the test method or contact time used. Iodophors had intermediate activity. Although they performed well against the virus, they failed to kill *M bovis* dried on plastic laminate surfaces, and they had low activity against the three EPA-specified bacteria. Isopropyl alcohol, alcohol mixtures, dilute glutaraldehyde, phenolics,

and quaternary ammonium compounds all failed to inactivate poliovirus, regardless of the contact time used. The glutaraldehyde and quaternary ammonium compounds also failed to kill the TB organism.

These data also showed: (1) disinfectants could kill the three EPA-specified organisms using the 10-minute EPA specified test (AOAC UDM) and not inactivate resistant organisms with clinical significance such as TB (dilute glutaraldehyde and quaternary ammonium compounds) and poliovirus (isopropyl alcohol, isopropylethyl alcohol mixtures, dilute glutaraldehydes, phenolics, and quaternary ammonium compounds); (2) disinfectants that killed the TB organism did not always inactivate poliovirus (isopropyl alcohols, isopropylethyl alcohol mixtures, and phenolics); (3) poliovirus was resistant to inactivation by several types of disinfectants regardless of contact time (isopropyl alcohol, dilute glutaraldehyde, phenolics, and quaternary ammonium compounds).

Figure 3 shows the performance of each of the 39 disinfectants that were included in the means reported in Figure 2. The data range within each of the eight disinfectant categories is also apparent.

Antimicrobial activity in the presence of bioburden

Table 3 shows results of CRA EWM and suspension tests performed on the 11 disinfectants selected for testing in the presence of bioburden. These data illustrate the adverse effect of whole blood on disinfectant antimicrobial activity. Tests with eight of the 11 disinfectants produced TNC counts when blood was added to the cultures. Only Citrace, Lysol sprays, and 70% vol/vol

denatured ethyl alcohol had consistently high antimicrobial activity across all five test organisms – both in the absence and presence of bioburden. Figure 4 gives a graphic representation of the data in Table 3.

In Figure 5, the 11 disinfectants listed in Table 3 have been grouped by active ingredient to display effects of the different types and concentrations of bioburden (10% and 50% human whole blood and 5% horse serum) used with four different test methods. Overall, the ethyl alcohol category performed best regardless of test method, type or concentration of bioburden, or test organism used. The chlorine category showed high activity against poliovirus, but activity against *M bovis* and *S aureus* was dependent on type and concentration of bioburden used. Whole blood (50%) caused a significant decrease in antimicrobial activity of chlorines, whereas horse serum (5%) did not interfere with antimicrobial activity. Both brands of iodophor had very low activity across all five test organisms in the presence of all types of bioburden. Figure 6 shows the appearance of CRA EWM trays and the EPA TB ATM filters after treatment with Lysol sprays, and Biocide and Wescodyne iodophors.

Figure 7 shows results of tests performed with and without 7.0 mol/L urea, which was used to dissociate aggregates formed when ethyl alcohol products interacted with whole blood bioburden. Separate assays were performed both with the aggregates intact and after dissociation by urea. The goal was to determine if $\log_{10}$ reductions were caused by disinfectant kill or entrapment of viable organisms within the aggregates. The data in Figure 7 indicate entrapment of viable organisms was not generally a problem. Overall, $\log_{10}$ reductions after urea

treatment were equal to or higher than tests not including urea, indicating that these ethyl alcohol disinfectants penetrated the whole blood and inactivated the organisms within.

Antimicrobial activity related to contact time

Figure 8 shows effects of 3-minute versus 10-minute disinfectant contact times in the absence and presence of human whole blood. Generally, increasing contact time made little or no difference in the antimicrobial activity of the four disinfectants tested. Both Biocide and Wescodyne iodophors had almost no disinfectant activity in the presence of blood, even when 10-minute contact times were used. On the other hand, Citrace and Lysol sprays produced greater than 3 $\log_{10}$ reduction for all five test organisms both at 3- and 10-minute contact times and in the absence and presence of whole blood. Therefore, Citrace and Lysol spray were selected for testing at shorter contact times of 2 and 1 minutes.

Figure 9 shows the rapid antimicrobial activity of Citrace and Lysol sprays. In the absence of whole blood, they produced $\geq \log_{10}$ reduction of all three test organisms in 1 minute. With whole blood present, both disinfectants produced $\geq 2.8 \log_{10}$ reduction in 1 minute. With increased contact time, a general increase in kill was achieved.

Antimicrobial activity related to wipe material

Figure 10 shows results from tests of the hypothesis that cotton in gauze sponges used to wipe surfaces interferes

with the antimicrobial activity of iodophors.[8] These data show that iodophor antimicrobial activity was inhibited by paper towels, but cotton and rayon/polyester had no adverse effect at the 3- minute contact time.

Tests for chemical interference and neutralizer efficacy

Table 4 shows results of work performed to test for chemical interference of residuals from ETO sterilization and/or materials used to construct trays used in the CRA EWM. Colony counts of the three test organisms showed inhibition of less than 6 CFU compared with control counts, which indicates that no toxic effects resulted from either variable.

Table 5 reports on efficacy of the neutralizers used in the CRA EWM procedure. The data show adequate neutralization of all products except quaternary ammonium compounds. Although residual activity was present to a small degree with quaternary ammonium compounds, it was not considered a problem because even with this advantage, these products failed to inactivate *M bovis* in the absence of bioburden.

Reproducibility of the wipe test

Reproducibility of the viable organism challenge on CRA EWM rays was demonstrated by the similarity of numbers of organisms computed from water control trays. The mean $\log_{10}$ challenges of 52 (without blood) and 34 (with blood) TB water control trays were 6.01 ± 0.22 and 6.57 ± 0.15, respectively.

Table 6 shows results of reproducibility tests performed on the CRA EWM. Average $\log_{10}$ reductions for *M bovis* achieved by two technicians using Clorox 1:5 in three test replications are shown. These data were consistent both within and between technicians.

Discussion

The goal of this investigation was to identify environmental surface disinfectants that had broad-spectrum antimicrobial activity, rapid action, and effectiveness both in the absence and presence of bioburden. Only Citrace, Lysol sprays, and 70% vol/vol denatured ethyl alcohol met the criteria, regardless of the test method or contact time used. Chemically, the two commercial products are similar. Citrace contains 66.6% wt/wt denatured ethyl alcohol (SDA-40-1), 0.12% ortho-phenylphenol, sodium nitrite rust inhibitors, proprietary deodorizer, and 3.5% hydrocarbon propellant (personal communication, Calvin Goeder, Caltech Industries, 1988) and Lysol sprays contain 79.0% wt/wt denatured ethyl alcohol (SDA-40-1), 0.1% ortho-phenylphenol, rust inhibitors, N-alkyl-N-ethyl morpholinium ethylsulfate deodorizer, and carbon dioxide propellant (personal communication, Joe Rubino, MS, Lehn and Fink, 1988).

Concentration of ethyl alcohol appeared to be a critical factor. Other products with formulations similar to Citrace and Lysol sprays, but containing less ethyl alcohol (CoeSpray with 53.5% wt/wt ethyl alcohol and Pro-Cide ES with 52.8% wt/wt ethyl alcohol), failed to inactivate poliovirus in the presence of bioburden (Table

3). These findings led to special tests performed to investigate the antiviral activity of various concentrations of SDA-40-1 denatured ethyl alcohol used in Citrace and Lysol sprays in the presence of 10% whole blood. Results showed a dramatic increase in virucidal activity with alcohol concentrations equal to or greater than 70% wt/wt. Klein and Deforest[52] reported similar results with poliovirus I. Also, many other investigators have reported very rapid inactivation of both viruses and bacteria with ethyl alcohol in concentrations of 70%-95%.[32,57-65] However, clinicians and researchers can be misled about alcohol concentrations in commercial products if they do not understand the volume/volume and weight/weight designations.

Antimicrobial activity of high concentration ethyl alcohol can become unpredictable if storage conditions allow undetected volatilization, environmental use conditions cause extremely rapid evaporation, or interfering denaturing agents are used. Unfortunately, clinicians have no way to monitor these variables. Therefore, Citrace and Lysol sprays appeared better suited than ethyl alcohol alone for environmental surface disinfection in clinical settings because they are sealed in airtight cans to prevent volatilization during storage; they contain other ingredients in their formulations that delay evaporation during use; and their denaturing agents are standardized.

Currently, official agencies and others recommend use of iodophors,[42,66-71] chlorines,[5,42,66,69,72-75] and phenolics[75] for disinfection of environmental surfaces. Results from this investigation suggest further review of these recommendations. This work showed iodophors had very poor antimicrobial activity in the absence and

presence of bioburden, regardless of the test method or contact time used. Horse serum and paper towel material caused iodophors to lose almost all activity. Many others have reported problems with iodophor antimicrobial activity on inanimate surfaces.[52,63,76-81] With chlorines, the potential of bioburden to diminish antimicrobial activity has been mentioned by many investigators[52,63,78,82] and this problem was further demonstrated in this study. The failure of different phenolic formulations to inactivate poliovirus using both 3- and 10-minute contact times was also demonstrated, and this problem has been noted by others.[6,63,83-85]

In addition, this study confirmed previous reports citing inability of isopropyl alcohol to inactivate poliovirus,[52,86] problems with antimicrobial activity of dilute glutaraldehyde,[87] and inability of quaternary ammonium compounds to inactivate poliovirus[52,83,84] and the TB organism.[78,88]

Precleaning of surfaces before disinfectant use has been stressed.[41,70,71,73,89-94] In the past, detergents have been preferred for this process. The obvious intent is to decrease proteins and other debris that interfere chemically with the antimicrobial activity of disinfectants. Although theoretically this appears sound, use of cleaners with low antimicrobial activity before disinfectant application causes cleaning personnel to touch concentrated body fluids containing potential pathogens. It ignores the fact that the wiping action can spread material from smaller concentrated areas to larger areas, and onto the wipe material and the person performing the cleaning. Now that disinfectants have been identified that penetrate and kill microbes within heavy bioburden, it

seems prudent to apply these agents first to a lower organism loads before human contact. Disinfectants containing high ethyl alcohol and ortho-phenylphenol can be used first to preclean and the disinfect in the following regimen: wet surface well and allow 2-3-minute disinfectant contact time to lower viable microbial load within debris; wipe vigorously to clean surfaces; rewet surface and allow 2-3-minute contact with disinfectant after cleaning. Appropriate barriers should be worn by cleaning personnel when using the suggested method.

Four clinically relevant points were demonstrated in this study:

– *Disinfectants often have selective kill.* Although clinicians have been advised that tuberculocidal products can be depended on to kill other important pathogens,[95] this is not necessarily true. In this study, for example, 70% vol/vol isopropyl alcohol and the phenolic, Sporicidin Spray, produced profound kill of the TB test organism (*M bovis*), but failed to inactivate a resistant nonenveloped virus (poliovirus). This questions the assumption of broad-spectrum kill, based solely on any one organism.

– *Dilution generally decreases disinfectant activity.* This effect is seen in both chlorine and iodophor categories (Table 2). Although there are reports indicating dilution increases the antimicrobial activity of iodophors,[96] this study showed activity decreased as dilution was increased from 1:106 up to 1:213. The same was true of chlorines as dilution was increased from 1:5 up to 1:20.

– *Bioburden affects disinfectants adversely.* The deleterious effect of bioburden on antimicrobial activity was demonstrated repeatedly and with all disinfectants in this

study to varying extents. Citrace, Lysol sprays, and 70% vol/vol denatured ethyl alcohol were affected least.

– *Many commercial products have marginal activity.* This point was illustrated by the fact that 28 of the 39 products in this study failed one or more test organisms even in the absence of bioburden. Only three of the 11 products tested in the presence of bioburden inactivated all five test organisms, regardless of the test method used.

Reproducibility of the AOAC Use Dilution Method has been debated for many years. In this investigation, the 60-tube version of this test was performed 230 times on 39 different products representing eight different active ingredients. When results were arranged in order of disinfectant major ingredient (Table 2), it became apparent that the AOAC UDM was reproducible when disinfectants with definite high antimicrobial activity were tested. However, when disinfectants with marginal activity were tested, variability of results increased substantially. To see this pattern, it was necessary to test several representative products from each of eight major active ingredients.

Conclusions

Data from this investigation indicated that optimum disinfection of environmental surfaces was highly formulation dependent. Of the 39 products tested, only three inactivated all five test organisms, regardless of test conditions. The other products showed deficiencies that contraindicate their use, in the formulation tested, as environmental surface disinfectants in clinical dental settings.

Information about the manufacturers of the products mentioned in this article is available from the authors. Neither the authors nor the American Dental Association has any commercial interest in the products mentioned.

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Table 1 — Alphabetical listing by brand name of all disinfectants tested. The far right column indicates the concentration of active ingredients at the recommended use-dilution listed on the label.

BRAND NAME	RECOMMENDED DILUTION	USE CONCENTRATION OF ACTIVE INGREDIENT(S)
1. BASIC-G	1:256	0.05% quaternary ammonium compounds 0.01% tetrasodium EDTA 0.001% essential oils
2. BIOCIDE	1:213	82 ppm available iodine
3. BIOCIDE	1:106*	164 ppm available iodine
4. BORAXO	Use as packaged	9.13% tetrasodium EDTA 5.0% isopropyl alcohol 0.07% 5-chloro 2 (2,4-dichlorophenoxy) phenol
5. CITRACE	Use as packaged	66.6% ethyl alcohol 0.12% o-phenylphenol
6. CLOROX	1:5	1.05% sodium hypochlorite
7. CLOROX	1:10	0.52% sodium hypochlorite
8. CLOROX	1:20	0.26% sodium hypochlorite
9. CLOROX FRESH SCENT	1:5	1.05% sodium hypochlorite
10. CLOROX FRESH SCENT	1:10	0.52% sodium hypochlorite
11. CLOROX FRESH SCENT	1:20	0.26% sodium hypochlorite
12. COESPRAY	Use as packaged	53.46% ethyl alcohol 0.9% essential oils 0.176% o-phenylphenol 0.044% p-tert-amylphenol
13. DENTASEPTIC	1:32	0.28% o-phenylphenol 0.03% o-benzyl-p-chlorophenol
14. DISPATCH	Use as packaged	0.55% sodium hypochlorite
15. ETHYL ALCOHOL (non-denatured)	Use as packaged	70% v/v non-denatured ethyl alcohol
16. ETHYL ALCOHOL (denatured)	Use as packaged	70% v/v denatured ethyl alcohol
17. ETHYL:ISOPROPYL ALCOHOL MIX	Experimental	40% ethyl alcohol 40% isopropyl alcohol
18. ETHYL:ISOPROPYL ALCOHOL MIX	Experimental	45% ethyl alcohol 45% isopropyl alcohol
19. EXSPOR	1:1:4	1.43% organic acid 0.23% sodium chloride
20. HIBISTAT TOWELETTE	Use as packaged	70% isopropyl alcohol 0.5% chlorhexidine gluconate
21. HI-TOR	1:256	0.06% quaternary ammonium compounds
22. ISOPROPYL ALCOHOL	Use as packaged	70% v/v isopropyl alcohol
23. LYSOL LIQUID (Pine Scent)	1:103	0.16% soap 0.05% pine oil 0.04% o-benzyl-p-chlorophenol 0.015% isopropyl alcohol 0.007% tetrasodium EDTA
24. LYSOL SPRAYS (Regular Scent, Fresh Scent ... Light Scent, Professional Lysol)	Use as packaged	79% ethyl alcohol 0.1% o-phenylphenol
25. MATAR	1:256	0.04% o-benzyl-p-chlorophenol 0.02% o-phenylphenol 0.01% isopropyl alcohol 0.01% p-tert-amylphenol 0.007% tetrasodium EDTA
26. MULTICIDE	1:32	0.28% o-phenylphenol 0.03% o-benzyl-p-chlorophenol
27. OMNI II	1:32	0.28% o-phenylphenol 0.03% o-benzyl-p-chlorophenol
28. PERMACIDE 18	Use as packaged	18% ethyl alcohol 18% isopropyl alcohol
29. PERMACIDE 30	Use as packaged	30% ethyl alcohol 30% isopropyl alcohol
30. PRECISE	Use as packaged	0.37% o-phenylphenol
31. PRESEPT LIQUID	Use as packaged	70% v/v ethyl alcohol B 1.25% w/v chlorhexidine gluconate 0.1% w/v quaternary ammonium compounds
32. PRESEPT TABLET	1:250	1000 ppm available chlorine
33. PRESEPT WIPE	Use as packaged	70% denatured ethyl alcohol 1.25% chlorhexidine gluconate 0.1% quaternary ammonium compounds
34. PROCIDE ES	Use as packaged	52.79% ethyl alcohol 0.176% o-phenylphenol 0.044% p-tert-amylphenol
35. SPORICIDIN SPRAY	Use as packaged	18.0% ethyl alcohol 1.41% phenol 0.25% essential oils 0.24% sodium phenate
36. STERALL SPRAY	Use as packaged	1.50% triethylene-glycol 0.25%glutaraldehyde
37. VITAWIPES	Unknown	10.28% quaternary ammonium compounds
38. WESCODYNE	1:213	75 ppm available iodine
39. WESCODYNE	1:106*	150 ppm available iodine

*Tested at twice manufacturer's suggested strength.

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ENVIRONMENTAL SURFACE DISINFECTANTS

Disinfectants for environmental surfaces must kill *rapidly & thoroughly.* To identify products meeting these requirements, CRA is purchasing & testing disinfectants from all over the world. Following chart summarizes 24 alcohol-based products where only 4 passed all tests, 9 chlorine-based products where only 2 passed all tests, 1 cleaner which did not pass all tests, 5 iodophore-based products where none passed all tests, 10 phenolic-based products where none passed all tests, 1 quaternary ammonium chloride-based product which did not pass all tests, and 9 wipes where only 1 passed all tests.

MATERIALS & METHODS.

Tuberculosis bacteria & polio virus are used as test organisms because they are human pathogens with known resistance to kill by many disinfectants. Results are reported as $\log_{10}$ reductions of 10^7 test organisms exposed to disinfectants for 1, 3, & 10 minutes using 3 different tests performed in triplicate, without & with fresh human whole blood present. By convention, less than a 3 $\log_{10}$ reduction constitutes inadequate kill. Full methods are described in following publication: Christensen, R.P., et al., JADA '89; 119:493-505.

BLACKENED SQUARES ON CHART INDICATE INADEQUATE KILL

	DISINFECTANT NAME	COMPANY	COUNTRY	MAJOR ACTIVE INGREDIENT(S)	TEST TIMES	POLIO VIRUS I (Mahoney) SUSPENSION TEST WITHOUT BLOOD	POLIO VIRUS I (Mahoney) SUSPENSION TEST WITH BLOOD	TUBERCULOSIS ORGANISM (M. Bovis) SUSPENSION TEST WITHOUT BLOOD	TUBERCULOSIS ORGANISM (M. Bovis) SUSPENSION TEST WITH BLOOD	TUBERCULOSIS ORGANISM (M. Bovis) SURFACE TEST WITHOUT BLOOD	TUBERCULOSIS ORGANISM (M. Bovis) SURFACE TEST WITH BLOOD
	ALCOHOL-BASED										
	1. AMCIDE	Ashland	USA	62.8% Ethyl alcohol (w/w)	1 min	0.0	0.0	>5.0	4.8		
					3 min	1.0	0.1	>5.0	5.0	>5.8	4.3
					10 min	2.5	0.3	>5.0	4.8		
	2. ASEPTI-PHENE RTU	Huntingdon Laboratories	Canada	66% Ethyl alcohol (w/w)	1 min	0.8	0.1	4.8	4.1		
					3 min	1.7	0.4	5.0	3.8	5.3	3.4
					10 min	2.7	0.5	>4.8	4.2		
	3. BACTOL BLUE	Whiteley Chemical	Australia	70% Ethyl alcohol (w/w)	1 min	1.0	0.1	>4.1	4.8		
					3 min	3.1	0.2	>4.1	4.9	>4.5	3.8
					10 min	3.7	0.3	>4.1	5.3		
*	4. BIOSURF	Micrylium Laboratories	Canada	70.5% Ethyl alcohol (w/w)	1 min	4.7	3.0	4.8	5.2		
					3 min	>4.8	>3.7	5.0	5.4	4.6	3.6
					10 min	>5.0	>3.8	4.9	5.4		
	5. CITRACE	Caltech Industries	USA	68.4% Ethyl alcohol (w/w)	1 min	4.4	0.3	>4.7	4.4		
					3 min	4.4	1.0	>4.7	4.4	5.0	3.8
					10 min	3.9	1.5	>4.8	4.8		
	6. CITREX	Caltech Industries	USA	68.1% Ethyl alcohol (w/w)	1 min	4.1	0.9	>4.8	>4.8		
					3 min	>5.0	1.8	>4.8	5.2	>5.1	3.8
					10 min	>5.0	2.3	>4.8	5.2		
	7. COE SPRAY	GC America	USA	63% Ethyl alcohol (w/w)	1 min	0.6	0.0	>5.0	5.4		
					3 min	1.3	0.3	>5.0	5.7	5.3	4.0
					10 min	2.6	0.1	>5.0	>4.8		
	8. COE SPRAY "The Pump"	GC America	USA	64% Ethyl alcohol (w/w)	1 min	0.6	0.0	5.2	5.0		
					3 min	1.7	0.2	>5.1	4.9	>5.1	4.0
					10 min	2.5	0.3	5.4	4.8		
	9. CROSS PREP	Cregine	Australia	70% Ethyl alcohol (w/v)	1 min	0.5	0.0	5.4	4.3		
					3 min	1.1	0.0	5.4	4.8	>4.8	3.6
					10 min	1.9	0.0	>5.1	4.8		
	10. FD 320	Orochemie	Germany & Canada	42% 1-propanol (w/v) 10% Ethyl alcohol (w/v)	1 min	0.0	0.0	5.4	3.7		
					3 min	0.0	0.0	5.4	5.0	>5.6	4.7
					10 min	0.3	0.0	>5.1	5.0		
	11. FD 322	Orochemie	Germany & Canada	22% 1-propanol (w/v) 39% Ethyl alcohol (w/v)	1 min	0.0	0.0	5.7	4.8		
					3 min	0.2	0.0	>5.1	>4.8	>4.8	4.5
					10 min	0.7	0.0	5.4	>4.7		
	12. FORMAL SPRAY	Courtin	England	43.5% Ethyl alcohol (w/v) 19.6% 2-propanol (w/v) 4.0% 1-propanol (w/v)	1 min	0.2	0.0	>5.1	5.8		
					3 min	0.6	0.0	5.4	>5.5	>5.9	3.3
					10 min	1.0	0.0	>5.1	>5.5		
	13. GENERIC RUBBING ALCOHOL	Bergen Brunswig Drug	USA	70% Isopropyl alcohol (v/v)	1 min	0.0	0.0	5.3	4.9		
					3 min	0.0	0.0	>5.0	>4.8	5.2	3.7
					10 min	0.0	0.0	>5.0	>4.8		
	14. GERMX	Germiphene	Canada	76% Ethyl alcohol (v/v)	1 min	4.1	1.5	5.1	4.5		
					3 min	4.1	1.6	5.1	4.7	4.9	3.6
					10 min	4.0	2.6	4.8	4.6		
*	15. GLEN 20	Reckitt & Colman	Australia	68% Ethyl alcohol (w/w)	1 min	>5.3	3.7	5.1	5.2		
					3 min	5.5	>4.0	5.0	5.3	5.4	3.3
					10 min	>5.5	3.9	4.4	5.5		
	16. INCIDIN SPEZIAL-SPRAY	Henkel Hygiene	Germany	38.4% Ethyl alcohol (w/w)	1 min	0.0	0.0	2.3	2.6		
					3 min	0.0	0.0	5.3	4.4	1.7	1.3
					10 min	0.1	0.3	4.9	>5.5		
	17. LYSOL SPRAY (Germicidal)	Reckitt & Colman	Canada	64% Ethyl alcohol (w/w)	1 min	>5.0	1.6	5.6	5.9		
					5 min	>5.0	2.2	5.4	5.5	5.6	3.6
					10 min	>5.0	2.6	5.3	5.8		
*	18. LYSOL I.C. SPRAY	ProBrands Division of Reckitt & Colman	USA	79% Ethyl alcohol (w/w)	1 min	>5.2	>3.5	4.8	>4.8		
					3 min	>5.3	>3.4	4.6	4.9	5.0	3.9
					10 min	>5.2	>3.4	4.8	>4.8		
*	19. LYSOL II SPRAY	Reckitt & Colman	USA	79% Ethyl alcohol (w/w)	1 min	>5.7	3.4	4.8	>4.7		
					3 min	>5.8	3.1	>4.8	>4.7	4.4	3.8
					10 min	>5.8	3.8	>4.8	>4.7		
	20. MIKROZID	Schülke & Mayr	Germany & England	35% 1-propanol (w/w) 23.5% Ethyl alcohol (w/w)	1 min	0.0	0.0	5.2	5.5		
					3 min	0.0	0.0	5.1	>5.2	5.8	5.3
					10 min	1.0	0.1	5.0	5.8		
	21. SAGROMED HBV	Schülke & Mayr	Germany	35% 1-propanol (w/w) 23.5% Ethyl Alcohol (w/w)	1 min	0.0	0.0	5.3	5.4		
					3 min	0.1	0.0	>5.1	>5.2	>5.4	4.5
					10 min	0.9	0.0	>5.1	>5.2		
	22. SEP-T-BAN	McCormick Scientific	USA	68.9% Ethyl alcohol (w/w)	1 min	4.8	0.7	5.4	4.1		
					3 min	>4.7	1.3	5.2	4.3	5.3	4.2
					10 min	>4.8	1.8	5.9	4.4		
	23. SURFEX-E	Microtek Associes	Canada	70% Ethyl alcohol (w/w)	1 min	>4.4	0.3	>5.1	>5.2		
					3 min	>4.8	1.6	>5.1	5.4	5.2	2.8
					10 min	>4.8	1.8	>5.1	>5.2		
	24. VIRAHOL	Veridien	USA	70% Isopropyl alcohol (w/w)	1 min	0.0	0.0	5.3	>5.3		
					3 min	0.0	0.0	>5.3	>5.3	4.6	3.6
					10 min	0.0	0.0	6.0	>5.1		
	CHLORINE-BASED	NOTE: All Clorox tests included both the Household and Institutional brands. Since results from the 8 tests were similar, they were combined.									
*	1-2. CLOROX 1:2 (Household & Institutional)	Clorox Company	USA	2.63% Sodium hypochlorite	1 min	>5.2	>4.8	>5.8	>5.2		
					3 min	>5.2	>4.8	>5.1	>5.2	6.1	5.5
					10 min	>5.2	>4.8	>5.1	>5.2		
	3-4. CLOROX 1:5 (Household & Institutional)	Clorox Company	USA	1.05% Sodium hypochlorite	1 min	>5.2	>4.5	>5.1	1.6		
					3 min	>5.4	>4.5	>5.1	4.0	6.5	1.8
					10 min	>5.3	>4.5	>5.1	5.1		
	5-6. CLOROX 1:10 (Household & Institutional)	Clorox Company	USA	0.53% Sodium hypochlorite	1 min	>5.2	4.5	5.7	0.6		
					3 min	>5.2	>4.8	>5.2	0.8	6.3	1.3
					10 min	>5.0	>4.8	>5.2	1.7		
	7-8. CLOROX 1:100 (Household & Institutional)	Clorox Company	USA	0.05% Sodium hypochlorite	1 min	>4.8	1.3	>5.2	0.5		
					3 min	>4.4	1.3	>5.2	1.0	2.7	0.1
					10 min	>4.4	1.4	>5.2	1.6		
	9. EXSPOR 4:1:1	Alcide	USA	0.26% Sodium chlorite	1 min	>5.3	>4.4	>5.0	>5.2		
					3 min	>5.1	>4.3	>5.0	>5.2	3.0	0.1
					10 min	>5.2	>4.3	>5.0	>5.2		

* at left of product name indicates product passed all tests

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BLACKENED SQUARES ON CHART INDICATE INADEQUATE KILL

DISINFECTANT NAME	COMPANY	COUNTRY	MAJOR ACTIVE INGREDIENT(S)	TEST TIMES	POLIO VIRUS I (Mahoney) SUSPENSION TEST WITHOUT BLOOD	POLIO VIRUS I (Mahoney) SUSPENSION TEST WITH BLOOD	TUBERCULOSIS ORGANISM (M. bovis) SUSPENSION TEST WITHOUT BLOOD	TUBERCULOSIS ORGANISM (M. bovis) SUSPENSION TEST WITH BLOOD	TUBERCULOSIS ORGANISM (M. bovis) SURFACE TEST WITHOUT BLOOD	TUBERCULOSIS ORGANISM (M. bovis) SURFACE TEST WITH BLOOD
CLEANER										
1. LYSOL I.C. FOAMING DISF/CLEANER not diluted	Reckitt & Colman	USA	0.1% Quaternary ammonium chloride	1 min	>4.8	0.3	0.1	0.0		
				3 min	>4.7	0.5	0.2	0.1	2.3	2.4
				10 min	>4.7	1.0	0.6	0.7		
IODOPHORE-BASED										
1. BI-ARREST (1:213)	Infection Control Technology	USA	75 ppm iodine	1 min	0.3	0.0	1.8	0.0		
				3 min	0.4	0.0	5.3	0.0	1.5	0.0
				10 min	1.9	0.1	4.8	0.0		
2. BIOCIDE (1:213)	Biotrol	USA	75 ppm iodine	1 min	0.3	0.0	1.8	0.0		
				3 min	0.4	0.0	5.3	0.0	1.5	0.0
				10 min	1.9	0.1	4.8	0.0		
3. IODOFIVE (1:213)	Cottrell, Ltd.	USA	75 ppm iodine	1 min	0.3	0.0	1.8	0.0		
				3 min	0.4	0.0	5.3	0.0	1.5	0.0
				10 min	1.9	0.1	4.8	0.0		
4. IODOPHOR DISINFECTANT (1:213)	Smart Practice	USA	75 ppm iodine	1 min	0.3	0.0	1.8	0.0		
				3 min	0.4	0.0	5.3	0.0	1.5	0.0
				10 min	1.9	0.1	4.8	0.0		
5. WESCODYNE (1:213)	Amsco	USA	75 ppm iodine	1 min	0.3	0.0	1.8	0.0		
				3 min	0.4	0.0	5.3	0.0	1.5	0.0
				10 min	1.9	0.1	4.8	0.0		
PHENOLIC-BASED										
1. ASEPTI-PHENE 128 (1:128)	Huntington	USA	6.00% o-phenylphenol 0.94% o-benzyl-p-chlorophenol 0.41% p-tert-amylphenol	1 min	0.0	0.3	1.2	0.3		
				3 min	0.0	0.3	4.8	2.6	3.7	0.5
				10 min	0.0	0.3	>4.9	>5.1	1.8	0.7
2. BI-ARREST II (1:256)	Infection Control Technology	USA	0.06% o-phenylphenol (K salt) 0.06% p-tertiary amylphenol (K salt)	1 min	0.0	0.2	1.8	0.4		
				3 min	0.0	0.3	5.0	3.0	4.0	0.9
				10 min	0.0	0.3	>4.9	>5.1	3.0	0.9
3. BIREX SE (1:256)	Biotrol Int'l.	USA	6.09% o-phenylphenol 6.03% p-tertiary amylphenol	1 min	4.1	0.3	0.2	0.0		
				3 min	>4.7	0.3	3.0	0.0	2.9	0.3
				10 min	>4.7	0.3	>5.1	0.0	1.1	0.2
4. LYSOL BULK (3:100)	Reckitt & Colman	USA	0.08% o-phenylphenol 0.08% o-benzyl-p-chlorophenol 0.66% other misc. active ingred.	1 min	0.0	0.2	1.4	0.0		
				3 min	0.0	0.3	5.4	0.2	4.1	1.1
				10 min	0.0	0.3	>5.1	0.9	4.6	0.9
5. LYSOL I.C.-DISINFECTANT (1:200)	ProBrands Division of Reckitt & Colman	USA	0.09% o-phenylphenol 0.09% o-benzyl-p-chlorophenol	1 min	0.0	0.9	2.1	0.1		
				3 min	0.0	0.7	>5.1	1.0	2.7	0.7
				10 min	0.0	0.7	>5.1	4.6	1.3	0.3
6. LYSOL I.C.-DISF/CLEANER (1:128)	ProBrands Division of Reckitt & Colman	USA	0.02% o-phenylphenol 0.06% o-benzyl-p-chlorophenol	1 min	0.0	0.7	0.8	0.2		
				3 min	0.0	0.5	4.5	2.2	3.3	1.1
				10 min	0.0	0.7	>5.2	5.1	0.7	0.6
7. LYSOL I.C.-PRESOAK not diluted	ProBrands Division of Reckitt & Colman	USA	0.55% o-phenylphenol 0.55% o-benzyl-p-chlorophenol	1 min	0.0	0.5	3.8	2.0		
				3 min	0.0	0.7	>5.2	4.4	>5.0	3.4
				10 min	0.3	0.7	>5.2	5.1	>5.1	4.9
8. PROCIDE SPRAY not diluted	Cottrell, Ltd.	USA	0.28% o-phenylphenol 0.03% o-benzyl-p-chlorophenol	1 min	0.0	0.3	0.9	0.0		
				3 min	0.0	0.4	5.4	0.4	3.8	0.9
				10 min	0.0	0.6	>6.2	3.0	2.8	0.7
9. SPORICIDIN not diluted	Sporicidin Int'l.	USA	1.86% Phenol 0.06% Sodium phenate	1 min	0.0	0.2	2.5	2.2		
				3 min	0.0	0.1	>5.2	4.2	>5.0	4.4
				10 min	0.1	0.2	>5.2	5.8	5.7	5.0
10. VITAL DEFENSE-D (1:32)	Block Drug	USA	0.28% o-phenylphenol 0.03% o-benzyl-p-chlorophenol	1 min	0.0	0.5	3.1	0.3		
				3 min	0.0	0.3	>6.2	3.2	4.4	1.8
				10 min	0.0	0.4	>6.2	>4.5	3.6	1.6
QUATERNARY AMMONIUM-BASED										
1. CAVICIDE	Karr	USA	0.25% Quaternary ammonium chloride 15.3% Isopropyl alcohol (w/w)	1 min			1.5			
				3 min	1.1	0.2	3.1	3.3	2.1	2.3
				10 min	>3.4	0.4	>5.5	>5.3		
WIPES										
1. ASEPTI-WIPE Pre-wetted	Ecolab Professional Products	USA	0.28% Quaternary ammonium chloride 8.0% Isopropyl alcohol (v/v)	1 min	0.0	0.2	0.0	0.0		
				3 min	0.1	0.0	0.0	0.1	0.0	0.1
				10 min	0.0	0.4	0.0	0.1		
2. METRIWIPES Pre-wetted	Metrex Research Corp.	USA	0.28% Quaternary ammonium chloride 8.0% Isopropyl alcohol (v/v)	1 min	0.0	0.2	0.0	0.0		
				3 min	0.1	0.0	0.0	0.1	0.0	0.1
				10 min	0.0	0.4	0.0	0.1		
3. SANI-CLOTH Pre-wetted	Nice-Pak PDI	USA	0.28% Quaternary ammonium chloride 8.0% Isopropyl alcohol (v/v)	1 min	0.0	0.2	0.0	0.0		
				3 min	0.1	0.0	0.0	0.1	0.0	0.1
				10 min	0.0	0.4	0.0	0.1		
4. DISCIDE ULTRA Pre-wetted	Palmero Healthcare	USA	0.34% Quaternary ammonium chloride 63.29% Isopropyl alcohol (w/w)	1 min	0.0	0.0	3.3	4.0		
				3 min	0.0	0.1	5.2	5.1	4.1	1.2
				10 min	0.0	0.1	>4.4	5.1		
* 5. INDI-WIPES (w/ Lysol Spray) † Not pre-wetted	Elrae Health Products	USA	3.17% Poly hydrochloride 7.11% Quaternary ammonium chloride	1 min	>3.8	>3.5	5.2	4.1		
				3 min	>3.5	>3.4	5.1	4.2	4.8	3.5
				10 min	3.7	>3.4	4.7	4.4		
† Indi-Wipes are a dry cloth impregnated with poly hydrochloride & quaternary ammonium chloride. The manufacturer's directions state, "Lightly dampen wipe with clean water or 70% alcohol." CRA tested the wipes by wetting with water or alcohol in separate tests. Results of wetting with 20 ml of water are below & results of a second group of tests wetting with 20 ml of Lysol Spray, whose active ingredient is 79% ethyl alcohol (w/w), are above. Commercially, there are no Indi-Wipes impregnated with Lysol Spray, but clinically they could be wetted thoroughly with Lysol Spray just before use & perform well.											
6. INDI-WIPES (w/ Water) † Not pre-wetted	Elrae Health Products	USA	3.17% Poly hydrochloride 7.11% Quaternary ammonium chloride	1 min	0.2	0.2	0.2	0.0		
				3 min	0.3	0.1	0.1	0.0	0.6	0.1
				10 min	0.3	0.2	0.2	0.0		
7. SPORICIDIN Pre-wetted	Sporicidin Int'l.	USA	1.86% Phenol 0.06% Sodium phenate	1 min	0.0	0.1	0.4	0.2		
				3 min	0.0	0.0	1.4	1.0	1.1	1.1
				10 min	0.7	0.0	4.9	3.9		
8. SUPER SANI-CLOTH Pre-wetted	Nice-Pak PDI	USA	0.5% Quaternary ammonium chloride 56% Isopropyl alcohol (w/w)	1 min	0.0	0.3	3.4	3.3		
				3 min	0.1	0.1	5.3	4.4	4.9	3.2
				10 min	0.0	0.1	5.1	4.4		
9. VIRAHOL Pre-wetted	Veridien Corp.	USA	79% Isopropyl alcohol (w/w)	1 min	0.0	0.1	4.7	4.5		
				3 min	0.0	0.0	4.7	4.7	1.5	0.1
				10 min	0.1	0.0	4.6	4.7		

* at left of product name indicates product passed *all* tests

CRA CONCLUSIONS: **Of 59 disinfectants tested only 7 passed all tests, & all of those were either ethyl alcohol or chlorine-based. Inability to inactivate a resistant virus was the most prevalent lack. Clinicians must be aware of kill potential of disinfectants, & avoid use of products that fail to kill resistant organisms from both bacterial & viral categories.**

1999

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REFERENCE NO. 16

Clinical Research Associates Abstract
J. Dent. Res. 69:379 #2161 March 1990

Tuberculocidal Activity of Glutaraldehyde & Glutaraldehyde/Phenol Disinfectants. R. Robison, D. Robinson, B. Ploeger, & R. Christensen (Clin. Res. As. Provo, UT USA)

Recent studies have established the inability of glutaraldehyde (glut) disinfectants to kill *Mycobacterium tuberculosis* (TB) rapidly. This deficiency has been diminished by raising the glut concentration from 2% to 3.2% & by addition of phenols. The purpose of this study was to compare the tuberculocidal activity of several glut & glut/phenol disinfectants. Products tested were: Cidex7; Cidexplus; Metricide Plus 30; ProCide 30; Sporicidin diluted 1:2, 1:8, & 1:16; Sterall undilute (UD) & 1:4; & Wipe Out UD & 1:2. The EPA Tuberculocidal Activity Test Method (quantitative) was used & linear regressions were performed to establish the time needed for a 6 $\log_{10}$ reduction of *M. bovis* (ATCC #35743) for each disinfectant. All products were tested once a week for 6 weeks to determine tuberculocidal activity & amount of glut & phenol present. TB kill time ranges from initial use to 6 weeks, ordered most to least effective, were: Sporicidin 1:2 = < 1 min; Wipe Out UD = 3 to 4 min; MetriCide Plus 30 = 21 to 28 min; Cidexplus = 22 to 42 min; Procide 30 = 28 to 42 min; Cidex7 = 29 to 58 min; Sterall UD = 36 to 42 min; Wipe Out 1:2 = 35 to 63 min; Sporicidin 1:8 = 53 to 58 min; Sporicidin 1:16 = 192 to 254 min; & Sterall 1:4 = 340 to 399 min. *It was concluded that the tuberculocidal activity of combination glut/phenol preparations was superior to glut-*

only preparations & water dilution recommended by manufacturers affected both types of formulations adversely.

REFERENCE NO. 17

VOLUME 15, ISSUE 5-- MAY 1991
CRA NEWSLETTER

SUBJECT: DISINFECTANTS, INSTRUMENT IMMERSION

Today heat sterilization is required for critical instruments because it provides best possible margin of safety against cross contamination. However, some items will not tolerate heat, & submersion in liquid disinfectant is highest level of treatment possible. Clinicians have difficulty selecting disinfectants because they have no way to know if expected microbial kill is achieved. Although agencies such as EPA, FDA, & ADA have responsibility to monitor claims, none actually *test* products. Their registration & approval programs are based on data submitted by manufacturers. CRA questioned accuracy of glutaraldehyde & glutaraldehyde-phenolic claims because apparently similar products had very different claims for test organism kill times, optimum dilution, & reuse life. Therefore, it was decided to perform tests to determine relevance of current label & promotional information on glutaraldehyde-based instrument immersion disinfectants. Following report includes: (1) goals of CRA testing; (2) results of testing; & (3) CRA conclusions.

1. GOALS OF CRA TESTING.

Purpose of tests was to determine of differences existed among several glutaraldehyde-based disinfectants. 10 formulations sold under 12 brand names were tested at various dilutions (total of 18 solutions). Tests included EPA Tuberculocidal Activity Test

Method (using *Mycobacterium bovis* ATCC #35743) to evaluate tuberculosis (TB) claims & a conventional suspension test (using poliovirus I, Mahoney strain) to evaluate poliovirus (polio) claims. These 2 organisms were selected because of their known resistance to inactivation by glutaraldehyde. Viruses of current interest to dental clinicians, such as HIV & HBV, were not used as test organisms because of technical difficulties they present in tests & because they have been shown to be readily susceptible to inactivation by most common disinfectants. Organic stress was not used because it is not required by EPA to validate organism claims.

2. RESULTS OF TESTING.

Chart on pages 2-3 gives details of this evaluation. Below is summary of results.

(a) TUBERCULOSIS TEST RESULTS – 12 of the 15 solutions with a TB kill claim required longer contact times than listed on their label to produce EPA required reductions.

(b) POLIOVIRUS TEST RESULTS – 10 of the 15 solutions with a polio claim required longer contact times than listed on their label to produce EPA required reductions.

(c) TUBERCULOSIS PLUS POLIOVIRUS TEST RESULTS – 4 of the 18 solutions tested fulfilled EPA requirements for both TB & polio through the last day of their reuse life claim in less than 30 minutes (Sporicidin 1:2 & 1:4, Cidex plus, &

Metricide Plus 30), & 7 more fulfilled these criteria in less than 60 minutes (Cidex 7, Procide, Metricide 28, Wipe Out not diluted, Banicide & Sterall not diluted, & Sporicidin diluted 1:8).

(d) TUBERCULOSIS KILL DID NOT NECESSARILY INSURE INACTIVATION OF POLIOVIRUS & VICE VERSA – Clinicians have been lead to believe kill of the TB organism signals kill of all other pathogens. CRA has reported previously that this is not true (Christensen, et al., JADA 119:493 Oct. '89). It is critical to note that 5 solutions in this evaluation required time beyond TB kill time to inactivate polio (Sporicidin diluted 1:16, 1:8, 1:4; Wipe Out not diluted; & Wipe Out diluted 1:2).

3. *CRA CONCLUSIONS:*

(a) Disinfectants with similar active ingredients had equivalent performance despite diverse label claims.

(b) Clinicians need to increase contact times well beyond conventional 10 minutes to assure inactivation of resistant organisms.

(c) Increased contact times were always associated with increased dilution of active ingredients.

(d) All 18 solutions tested inactivated both test organisms to levels required by EPA, if clinician is willing to tolerate whatever contact time data indicate. CRA recommendations on specific brand names tested are listed on page 3.

TIME REQUIRED TO KILL TUBERCULOSIS & POLIOVIRUS TEST ORGANISMS
CRA test results vs. manufacturers' stated times

CRA contact times are means of multiple tests performed over the solution's reuse life. Add at least 15 minutes to all means for margin of safety to allow for clinical-use stress on solutions (i.e. organic load, dilution, aging).

BRAND NAME, COMPANY, & COST/OZ AT DILUTION TESTED	TIME	DISINFECTANT CATEGORY	DILUTION TESTED BY CRA	PERCENT ACTIVE INGREDIENTS AT DILUTION TESTED	MFR. STATED REUSE LIFE
1. SPORICIDIN 1:2 ‡ Ash/Dentsply Cost/oz @ this dilution = $1.66	TB Mfr rec: none CRA test: 1 m Polio Mfr rec: none CRA test: 1 m	Glutaraldehyde-Phenolic	Diluted 1 part H_20 by CRA §	1.00% glutaraldehyde 3.53% phenol 0.60% sodium phenate	No stated reuse life for this dilution
2. SPORICIDIN 1:4 ‡ Ash/Dentsply Cost/oz @ this dilution = 83¢	TB Mfr rec: none CRA test: 6 m Polio Mfr rec: none CRA test: 11 m	Glutaraldehyde-Phenolic	Diluted 3 parts H_20 by CRA §	0.50% glutaraldehyde 1.76% phenol 0.30% sodium phenate	No stated reuse life for this dilution
3. METRICIDE PLUS 30 Metrex Cost/oz @ this dilution = 16¢	TB Mfr rec: 20 m CRA test: 23 m Polio Mfr rec: 3 m CRA test: 6 m	Glutaraldehyde	Used as packaged per mfr. directions	3.20% glutaraldehyde	30 days
4. CIDEXPLUS J&J Medical Cost/oz @ this dilution = 22¢	TB Mfr rec: 20 m CRA test: 26 m ¶ Polio Mfr rec: 10 m CRA test: 6 m	Glutaraldehyde	Used as packaged per mfr. directions	3.20% glutaraldehyde	28 days
5. WIPE OUT Biotrol, Int'l Cost/oz @ this dilution = 18¢	TB Mfr rec: 15 m CRA test: 4 m Polio Mfr rec: 10 m CRA test: 35 m	Glutaraldehyde-Phenolic	Used as packaged per mfr. directions	0.30% glutaraldehyde 1.00% phenol as sodium phenate	45 days
6. CIDEX 7 J&J Medical Cost/oz @ this dilution = 22¢	TB Mfr rec: 90 m CRA test: 35 m ¶ Polio Mfr rec: 10 m CRA test: 6 m	Glutaraldehyde	Used as packaged per mfr. directions	2.00% glutaraldehyde	28 days
7. METRICIDE 28 Metrex Cost/oz @ this dilution = 14¢	TB Mfr rec: 10 m CRA test: 36 m Polio Mfr rec: 10 m CRA test: 8 m	Glutaraldehyde	Used as packaged per mfr. directions	2.00% glutaraldehyde	30 days
8. BANICIDE Pascal Cost/oz @ this dilution = 28¢	TB Mfr rec: 30 m CRA test: 44 m Polio Mfr rec: 10 m CRA test: 44 m ¶	Glutaraldehyde	Used as packaged per mfr. directions	2.00% glutaraldehyde	42 days
9. STERALL Colgate/Hoyt Cost/oz @ this dilution = 22¢	TB Mfr rec: 30 m CRA test: 47 m Polio Mfr rec: 10 m CRA test: 43 m ¶	Glutaraldehyde	Used as packaged per mfr. directions	2.00% glutaraldehyde	42 days
10. PROCIDE Cottrell, Ltd. Cost/oz @ this dilution = 20¢	TB Mfr rec: 10 m CRA test: 48 m Polio Mfr rec: 10 m CRA test: 7 m	Glutaraldehyde	Used as packaged per mfr. directions	2.00% glutaraldehyde	28 days
11. SPORICIDIN 1:8 Ash/Dentsply Cost/oz @ this dilution = 41¢	TB Mfr rec: none CRA test: 50 m Polio Mfr rec: none CRA test: 53 m	Glutaraldehyde-Phenolic	Diluted 7 parts H_2O per mfr. directions	0.25% glutaraldehyde 0.88% phenol 0.15% sodium phenate	30 days
12. OMNICIDE Cottrell, Ltd. Cost/oz @ this dilution = 20¢	TB Mfr rec: 10 m CRA test: 64 m Polio Mfr rec: 10 m CRA test: 17 m	Glutaraldehyde	Used as packaged per mfr. directions	2.00% glutaraldehyde	28 days

13. GLUTAREX 3M Cost/oz @ this dilution = 29¢	TB Mfr rec: 10 m CRA test: 70 m Polio Mfr rec: 10 m CRA test: 8 m	Glutaraldehyde	Used as packaged per mfr. directions	2.00% glutaraldehyde	28 days
14. COLDSPOR 1:20 Metrex Cost/oz @ this dilution = 16¢	TB Mfr rec: 10 m CRA test: 1.9 h Polio Mfr rec: 2 m CRA test: 1.4 h ¶	Glutaraldehyde-Phenolic	Diluted 19 parts H_2O per mfr. directions	0.50% glutaraldehyde 0.025% o-phenylphenol 0.005% p-tert-amylphenol	30 days
15. WIPE OUT 1:2 Biotrol, Int'l Cost/oz @ this dilution = 9¢	TB Mfr rec: 15 m CRA test: 72 m Polio Mfr rec: 10 m CRA test: 2.8 h	Glutaraldehyde-Phenolic	Diluted 1 part H_2O per mfr. directions	0.15% glutaraldehyde 0.50% phenol as sodium phenate	45 days
16. SPORICIDIN 1:16 Ash/Dentsply Cost/oz @ this dilution = 21¢	TB Mfr rec: 10 m CRA test: 3.4 h Polio Mfr rec: 10 m CRA test: 3.5 h	Glutaraldehyde-Phenolic	Diluted 15 parts H_2O per mfr. directions	0.13% glutaraldehyde 0.44% phenol 0.08% sodium phenate	30 days
17. BANICIDE 1:4 Pascal Cost/oz @ this dilution = 7¢	TB Mfr rec: 30 m CRA test: 5.7 h Polio Mfr rec: 10 m CRA test: 5.0 h	Glutaraldehyde	Diluted 3 parts H_2O per mfr. directions	0.50% glutaraldehyde	21 days
18. STERALL 1:4 Colgate/Hoyt Cost/oz @ this dilution = 6¢	TB Mfr rec: 30 m CRA test: 6.4 h Polio Mfr rec: 10 m CRA test: 5.9 h ¶	Glutaraldehyde	Diluted 3 parts H_2O per mfr. directions	0.50% glutaraldehyde	21 days

‡ = More concentrated than recommended § = CRA tested solutions more concentrated than company recommends for products that did not received 6 $\log_{10}$ TB & 3 $\log_{10}$ polio reduction within recommended contact times. ¶ = Cidexplus & Cidex 7 TB claims by manufacturer are for 25°C, & Sterall & Banicide polio claims by manufacturer are for 23°C. Higher temperatures would result in shorter contact times.

CRA OBSERVATIONS & RECOMMENDATIONS

CRA OBSERVATIONS –

(1) POTENTIAL REASONS FOR DIFFERENT CLAIMS OF APPARENTLY SIMILAR PRODUCTS – (a) Different efficacy test methods & parameters used; (b) Different laboratory technicians with different techniques; (c) Error within test methods; (d) Standard tests may not have been performed properly i.e. improper neutralization, improper organism challenge, no temperature control, etc.; & (e) Present registration system does not provide confirmation of disinfectant efficacy.

(2) NEED FOR DISINFECTANT MONITORS – Routine use of disinfectant monitors is necessary to tell clinician to discard solution when active ingredients have dropped to independable levels.

(3) NO IDEAL DISINFECTANT – All disinfectants tested exhibited one or more characteristics that can be undesirable such as fair to poor odor, corrosion of metal if soaked overnight, staining of skin &/or plastic instruments, special requirements for disposal, high cost, etc. Ideal disinfectant lacking in all negative characteristics does not exist. Clinicians must determine characteristics they can & cannot tolerate.

(4) EFFECTS OF WATER DILUTION – Undesirable clinical characteristics & costs are decreased by water dilution, but dilution also decreased antimicrobial activity which extended contact times significantly.

(5) BENEFITS & PROBLEMS WHEN GLUTARALDEHYDE COMBINED WITH PHENOLIC – Combination of glutaraldehyde with phenolics is attempt to take advantage of glutaraldehyde's

rapid virucidal activity & phenolic's rapid tuberculocidal activity. CRA tests showed concentrations of glutaraldehyde & phenolic are critical. Altering concentrations of either chemical can skew activity toward one organism at expense of other.

(6) REUSE LIFE CLAIMS VS. SOLUTION LEVELS – Under heavy use, solution levels can drop so low after about 2 weeks that longer reuse life claims become irrelevant.

CRA RECOMMENDATIONS –

Since all 18 disinfectants tested eventually destroyed both test organisms, clinical choices can be based on desired *speed of disinfection, amount of tolerable active ingredients, & cost.* CRA suggests following rationale for choosing product best suited to individual needs.

(1) *IF CLINICIAN NEEDS FASTEST POSSIBLE KILL OF RESISTANT ORGANISMS & COST, ODOR, & CORROSION ARE NOT CONSIDERATIONS* – Sporicidin 1:2 or 1:4 are products of choice.

(2) *IF CLINICIAN NEEDS FAST KILL OF RESISTANT ORGANISMS & TOLERANCE OF ORGANIC LOAD OVER TIME IS NEEDED* – Metricide Plus 30 or Cidexplus 3.2% glutaraldehydes are products of choice. These higher concentration glutaraldehydes are ideal for *pre*-disinfection of instruments to lower microbe counts before handling. CRA tests show higher glutaraldehyde concentration maintains activity longer during heavy use & in presence of heavy bioburden.

(3) *IF CLINICIAN WILL TOLERATE 45-60 MINUTE SOAK TIME INDUSTRY STANDARD IS 2% GLUTARALDEHYDE* – There are numerous 2% glutaraldehydes & glutaraldehyde-phenolics from which to choose (Banicide not diluted, Cidex 7,

Procide, Metricide 28, Sporicidin diluted 1:8, Sterall not diluted, & Wipe Out not diluted). For practices that can tolerate soak times from 1-3 hours, 2 additional 2% glutaraldehydes (Glutarex & Omnicide) & 2 additional glutaraldehyde-phenolics (ColdSpor diluted 1:20 & Wipe Out diluted 1:2) are possibilities.

(4) *WHEN SOAK TIMES CAN EXTEND 4-6 HOURS & LOWEST POSSIBLE CONCENTRATION OF ACTIVE INGREDIENTS IS DESIRED TO MINIMIZE HYPERSENSITIVITY* – Sporicidin diluted 1:16 & Banicide & Sterall both diluted 1:4 fill this need.

REFERENCE NO. 18

JADA, Vol. 129, July 1998

HOW WELL DOES THE CHEMICLAVE STERILIZE HANDPIECES?

ROBERT A. KOLSTAD, PH.D.

ABSTRACT

Using the Food and Drug Administration's protocol for testing health care sterilizers, the author investigated the ability of chemical vapor and steam to sterilize handpieces. Five internal sites of six high-speed handpiece models and four internal positions of one low-speed handpiece model were each inoculated with 10^6 *Bacillus stearothermophilus* spores. Half-cycle challenges were conducted with Chemiclave models EC 5500 and 8000 (Barnstead/Thermolyne) and with two autoclaves, Tuttnauer 2540M (large chamber) (Tuttnauer USA Co., Ltd.) and Statim Cassette (SciCan USA). Experiments with spores either openly exposed or partially enclosed prove that

Between-patient instrument processing – including cleaning, packaging, loading, device operation, monitoring and storage – is a subject of continuous interest to heath care practitioners.[1-6] So, too, is sterilization, which dental offices experience as procedure, not end effect. The practicing dental community does not inspect instruments cycled through steam, heated air or chemical vapor for surviving microorganisms.

The marketing of sterilization devices is regulated by the Food and Drug Administration, or FDA. In 1976, that

agency began developing an official definition of sterilization, requiring manufacturers intending to market health care sterilization devices to prove efficacy claims.[7] The FDA published its requirements in 1993.[8] In that document, the agency prescribed the boundaries of the sterilization process, incorporating two basic principles.

– **Overkill.** After use, each instrument is assumed to be contaminated with microbes higher in both number and resistance than those actually encountered. Accordingly, efficacy studies are to be initiated with one million (10^6) microbes of the kind most difficult to destroy by the lethal agent in question. For example, bacterial spores could be used in a study of the effectiveness of steam or heated air.

– **Sterility assurance level.** The sterility assurance level, or SAL, is the probability – by consensus considered low enough for patient safety – that each processed item bears a single surviving microbe. For reused health care instruments, the SAL is one in one million (10^{-2}).

Sterilization claims are expressed as the stress needed to achieve the prescribed viability reduction – for example, the length of time an instrument is exposed to a particular temperature. In practice, microbes on suitable carriers – such as stainless steel washers – are exposed to steam, heated air or another lethal agent. The sterilization claim is the time during which the population is reduced to 10 percent multiplied by 12 (the time during which viability would fall from 10^6 to 10^{-6}). In turn, that rate of extrapolation must be confirmed with a test employing instruments that bear 10^6 microbes. The exposure time

during which total kill is achieved must not exceed half the sterilization claim.

In 1995, the FDA embarked on an initiative – still in draft form[9] – complementary to the 1993 ruling. It stated that handpiece manufacturers must validate sterilization claims through microbial testing closely related to confirmatory challenges mandated in the 1993 publication. This study was conducted to determine the ability of chemical vapor and steam to sterilize rotary instruments according to the FDA handpiece testing protocol.

MATERIALS AND METHODS

Microbial materials. I obtained *Bacillus stearothermophilus* American Type Culture Collection, or ATCC, 7953 spores in aqueous suspension from SPS Corp., $2x10^8$/milliliter $D_{121\ C}$=1.9 minutes tested on a paper carrier.

The incubation medium I used was Trypticase Soy Broth (Becton-Dickinson), or TS broth. Sporulation medium consisted of Nutrient Broth (Difco Laboratories) plus 0.005 grams per liter of manganese sulfate solidified with 2 percent agar 20 mL per petri plate.

Equipment. Six each of six high-speed handpiece models were used: Midwest Tradition L and Midwest Quiet Air (Dentsply Midwest), Lares 557 and Lares 757 (Lares Research), KaVo 642B (KaVo America Corp.) and Star 430SWL (Den-Tal-Ez Inc.). Twelve low-speed handpieces Star 62398 (Det-Tal-Ez Inc.)were employed. All handpieces were drawn from manufacturers' new inventory.

The chemical vapor sterilizers I used were Chemiclave EC 5500 (representing the nonvacuum line) and Chemiclave 8000 (prevacuum/post vacuum line). The lethal agent Vaposteril (Barnstead/Thermolyne), which I used in both chemical vapor sterilizers, includes the active ingredients ethanol 72.38 percent and formaldehyde 0.23 percent. For all Chemiclave models, the manufacturer's stated maximum temperature is 132 C. With the aid of an Omega model COCO001 thermocouple (Omega Engineering Inc.), I validated that temperature. Pressurized steam equipment included the large-chambered Tuttnauer 2540M (Tuttnauer USA Co., Ltd.), whose operational temperature – 121 C and 134 C – were adjusted per thermocouple, and the small-chamber Statim Cassette autoclave (SciCan USA), whose operational temperature – 135 C to 137 C – could be thermocouple-measured but not adjusted.

For biological challenges, the Chemiclaves were preheated with a dummy cycle, to minimize microbial destruction during warmup. Timing began when chamber pressure reached 20 pounds per square inch. I also ran a dummy cycle with the Statim autoclave. Timing began at 135 C. In contrast, I did not prewarm the Tuttnauer autoclave. The Tuttnauer's timing begins when the chamber door is locked; thus, microbes are exposed to the lowest total lethal exposure when the device starts out "cold".

Research design. The research design was developed in accordance with the FDA's Guidance Document on Dental Handpieces.[9] The FDA's designated test organism

for steam is *B. Stearothermophilus* ATCC 7953. For Chemiclave validation, the FDA makes no specific recommendation. However, based on a study that compared three bacterial species' resistance to heated Vaposteril,[10] the Chemiclave manufacturer selected *B. Stearothermophilus* as the test microorganism. As mentioned earlier, I used *B. Stearothermophilus* ATCC 7953 for Chemiclave challenges.

The FDA handpiece document calls for inoculating unsealed internal locations with 10^6 microbes each, after which inoculated instruments are exposed to one-half the nominal sterilization cycle. The minimum sample size is three handpieces per challenge run, with three runs per handpiece type.

Handpieces brought into the study from manufacturers' stock were clean; instruments in testing emerged from incubation either sterile or heavily contaminated with vegetative microbial growth. To ensure that all handpieces started out clean, I prepared them for experimental trials by exposing them to high-quality (97 percent dry) steam in a laboratory-grade autoclave, rinsed them in tap water, scrubbed them for three minutes in a Ney model 250 ultrasonic bath (Neytech Inc.) containing liquid detergent, rinsed them in distilled water and dried them.

High-speed handpiece trials. After initial sterilization and cleaning, handpieces were readied as though for clinical use, according to manufacturers' directions.

– The chuck, the drive air line and the exterior surfaces of the Midwest handpieces were sprayed with Midwest Plus Handpiece Cleaner. These handpieces were brush-scrubbed in running tap water, rinsed, run without water

until excess cleaner was expelled, then dried. They were lubricated with three drops of Midwest Plus Handpiece Lubricant that were placed in the drive air line. They then were run without water until excess oil was expelled.

– Lares handpieces were brush-scrubbed in running water, rinsed, dried, and cleaned and lubricated from the base with Lares Handpiece Conditioner. They were run 60 seconds with air and water. Lares handpieces were cleaned and lubricated both before and after heat exposure.

– KaVo handpieces were brush-scrubbed in running water, dried, and cleaned and lubricated from the base with KaVo Spray.

– Star handpieces were brush-scrubbed with isopropyl alcohol, then dried.

Contamination involved deposition of 0.01 mL spore suspension per site. A microsyringe was used for inoculation. In one experimental series (conducted with large lumens), turbines were removed and inoculated on one or more blades. In addition, drive air lines and exit air lines were inoculated. After the parts were dried for one hour at 37 C, turbines were returned to their housing. In an alternative test series (conducted with small lumens), chip air lines and cooling water lines were inoculated, then dried one hour at 37 C.

Contaminated handpieces were either subjected to no prechallenge handling or processed according to manufacturers' directions. Contaminated handpieces sealed individually in paper pouches obtained from the Chemiclave manufacturer were exposed to heat, three per

cycle. Three runs were conducted per handpiece type and protocol step. Half-cycles were 10 minutes (Chemiclaves), five minutes (Statim), 17.5 minutes (Tuttnauer low temperature) and 12.5 minutes (Tuttnauer high temperature).

After exposure, handpieces were immersed in 80 mL TS broth in 100-mL graduated cylinders and incubated at 56 C until growth appeared or seven days had elapsed (step H in Figure 1). Growth signifies recovery of one or more viable spores. To ensure that growth was *B. Stearothermophilus*, two cultural characteristics were controlled. During its multiplication in TS broth, *B. Stearothermophilus* ATCC 7953 displays uniform turbidity. As growth ceases, a white pellet gathers at the container base; the medium above is clear. Second, a sample of microbial suspension was spread on TS agar and incubated for 40 hours at 56 C. After this, vegetative rods, rods containing endospores and free spores could be observed through a phase-contrast microscope.

Low-speed handpiece trials. After their initial sterilization and cleaning, handpieces were inoculated at four positions, each with 0.01 mL spore suspension. Sites included the nose cone chuck; input drive air port, forward chuck rotation; input drive air port, reverse chuck rotation; and the exit air port. Inoculated motors were dried at 37 C for one hour.

To determine whether packaging affects sterilization, I exposed contaminated handpieces, some open and some sealed in pouches, to heat, three instruments per challenge. Three runs were conducted for each of the two packaging modes. The Statim autoclave and Chemiclave

model EC 5500 were preheated with dummy cycles. Timing began as in the high-speed handpiece trials. The Statim half-cycle for unpackaged loads is 105 seconds and for packaged items, five minutes. The Chemiclave half-cycle is 10 minutes.

After being exposed, handpieces were immersed in 32 mL TS broth in glass tubes 25 millimeters x 150 mm and incubated at 56 C until growth appeared or seven days had elapsed. Control for *B. stearothermiphilus* was conducted as in the high-speed handpiece trials.

Accidental contamination control. To demonstrate that microbes surviving heat exposure were not chance contaminants but were included in the deliberate inoculum, modified low-speed challenges were conducted. As a negative control, 12 handpieces were processed as outlined above. Six instruments were transferred to TS broth and incubated at 56 C for seven days. No growth ensued. The other six handpieces were packaged, then subjected to half-cycle exposure in Chemiclave model EC 5500, removed from packaging and incubated in TS broth for seven days. No growth ensued. In the positive control, 12 handpieces were sterilized and cleaned. The handpieces were sterilized again, inoculated, packaged and exposed to half-cycle Chemiclave vapor, then incubated. Eight incubation tubes showed growth, all typical of *B. Stearothermophilus*.

Washers. To independently investigate Vaposteril lethality and penetration, I conducted challenges with spores on exposed surfaces and deep within tubes. Stainless steel washers 13 mm in diameter were inoculated with 0.01 mL spore suspension. Samples were dried at 37

C for one hour, then exposed to heat in a Chemiclave model EC 5500. Timing commenced at 20 psi.

In one set of trials, five washers resting on a wire-mesh screen were subjected to one, two, three, four, and five minutes' exposure. In another challenge series with the same timings, five glass tubes 15 x 125 mm, each holding one washer at the bottom, were placed in the chamber with the open end at an angle 30 degrees above horizontal. Loaded tubes were not capped. Exposed samples were incubated in TS broth at 56 C for seven days.

RESULTS

High-speed handpieces: Chemiclave tests. Initial half-cycle exposure involving contamination of all five internal sites resulted in spore outgrowth. Succeeding trials, conducted to more closely identify any sites that might offer protection, differentiated large lumens (air drive, turbine, air exit) and small lumens (air chip line and cooling water line). Both Chemiclave models successfully destroyed the biological challenge in the air-propulsion tract. Model EC 5500 was less effective than model 8000.

In retrospect, lubrication played no role in the outcome; no surviving spores were recovered from lubricated surfaces.

High-speed handpieces: autoclave tests. Initial trials at all five loci with the Statim device resulted in no microbial growth. Complete testing – large vs. small lumens – with the Statim and Tuttnauer autoclaves, the latter at both 121 C and 134 C, resulted in no surviving growth.

Low-speed handpieces. Four openly accessible locations were inoculated, and I made no attempt to discover which could be protective. In each of the two Chemiclave challenges, seven instruments emerged bearing viable spores; packaging appeared to play no role. In contrast, the Statim autoclave fully destroyed the bioburden, both in the faster unpackaged mode and the packaged mode.

Washers. The high-speed handpiece test results prove that chemical vapor can kill *B. Stearothermophilus* spores, and they suggest that narrow passageways thorough which the lethal agent must traverse could restrict contact with the biological target. The washer test separated lethality and access. Metal was chosen as a carrier, because handpiece lines are so constructed and because the material basis of a carrier greatly influences resistance of borne spores.[11] Clearly, Vaposteril is an effective killing agent. At one-minute exposure all samples emerged sterile.

In the enclosure trials, open tubes were positioned close to horizontal to best allow lethal agent penetration. Even that measure failed to overcome access limitation. It should be noted that autoclave exposure destroys spores in open tubes. The tube experiments demonstrate a discontinuous relationship between time in device (a better term than "exposure time") and lethal effect; moreover, time in device necessary to achieve even a partial effect was very long, relative to open exposure efficiency.

DISCUSSION

This study demonstrates that chemical vapor is an excellent sporicidal agent whose lethal activity is throttled by

small high-speed handpiece lines and motor internal locations. Challenges conducted with contaminated washers – either openly exposed to the lethal agent or sequestered at the closed end of glass tubes – strongly support the thesis that when chemical vapor fails, that failure is attributable to weak penetration, not killing power. So does experience with sterilizer operation. Considerably more time (and heat) is required to convert water to steam than to convert liquid Vaposteril to vapor. The higher specific heat of steam likely accounts for its greater penetration.

Well before the FDA began registering health care sterilizers, the Chemiclave was approved for marketing by the Environmental Protection Agency, which requires that equipment discharging potentially hazardous chemicals (in this case, formaldehyde) pass a stringent biological challenge involving spores of two bacterial genera borne on both hard and soft surfaces.[12] The hard carrier – small porcelain cylinders – is meant to represent instrument surfaces. Somehow the critical distinction between surfaces and instruments became lost; thus was born the myth that Chemiclaves sterilize all dental instruments. Downstream, that unproven assertion was to be recited as fact in risk management courses, journal and trade articles, infection control books, dental school lectures, expert conferences and other venues. The federal services, military and civilian, did decertify Chemiclaves, but the reason was formaldehyde toxicity. Handpiece manufacturers provide strong backing for the use of Chemiclaves by recommending it in package inserts. In fact, those manufacturers have operational integrity in mind, not pathogens: users are warned against using heat

beyond 135 C. Finally, endorsement for Chemiclaves comes from sterilization monitoring services, which unwittingly lead their subscribers to believe that spore-strip or other biological-indicator destruction signals load sterility.

The fundamental concept of health care sterilization shifted from surrogates to instruments when the FDA issued requirements for sterilizer marketing. That agency prohibits manufacturers from making claims for instruments without direct proof. Chemiclave models 7000 and 8000 are FDA-registered for handpiece sterilization, but those models are no longer sold. Chemiclaves now on the market are registered with the FDA, but without handpiece claims. The FDA does not specify instruments to be included in certification. Moreover, the agency prohibits manufacturers from informing the practicing dental community that their devices are FDA-registered. Obviously, that subtlety is lost on the practicing dental community, which cannot be expected to decipher arcane interplay between government and industry.

CONCLUSION

This study should have an impact on dental sterilization policy. For offices in which the sole sterilizer is a Chemiclave, I advise ordering and autoclave for high-speed handpieces and low-speed attachments. Motors can be protected with plastic sleeves and autoclaved periodically. Reusable three-way syringe tips are best replaced with disposable tips. Until the autoclave arrives, a pressure cooker operated with distilled water should suffice for sterilizing handpieces and motor attachments.

For its part, the Chemiclave manufacturer might wish to enhance chemical vapor penetration, either by modifying the Vaposteril formula or by redesigning the equipment and its operation.

This study was conducted with testing less stringent than that required for FDA sterilizer certification. I endorse no equipment. In selecting an autoclave, the practitioner should verify that the device has been registered with the FDA and bears a claim for whatever instrument the practitioner recognizes as most difficult to sterilize. Generally, this means high-speed handpieces.

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